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

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ABSTRACT
Objective – the aim of this article was to demonstrate a new concept in the etiology of bilirubin-induced neurologic dysfunction (BIND) and highlight the role of D-Penicillamine (D-PA). Study Design – The authors conducted a review searching the literature of gasotransmitters and of neuroprotective effects of D-PA in the neonatal period. Results – nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H2S) are regarded as gasotransmitters and endogenous neuromodulators playing multiple roles in CNS under physiological and pathological states. D-PA by its ability to modulate both oxidative stress and NO pathway may have significant neuroprotective effects in cases jeopardized by BIND or retinopathy of prematurity (ROP). Other endogenously generated small-molecule species, such as CO and H2S have also been shown to possess important signaling properties. Interpretation – The present research article address the medical necessity of the use of D-PA as a neonatal neuroprotective drug.

KEYWORDS: Bilirubin-induced neurologic dysfunction; Reactive oxygen species; nitric oxide; carbon monoxide; hydrogen sulfide; D-Penicillamine in the neonatal period.

ABBREVIATIONS
AD - Alzheimer disease; BG - Basal ganglia; BIND - Bilirubin-induced neurologic dysfunction; CO - Carbon monoxide; BPD - Bronchopulmonary dysplasia; CNS - Central nervous system; CTRI - Copper transporter 1; D-PA - D-Penicillamine; H2S - Hydrogen sulfide; HO - Heme oxygenase; MD - NHBI - Neonatal hyperbilirubinemia; ROP - retinopathy of prematurity; ROS - Reactive oxygen species; TB - Total bilirubin level; TBI - Traumatic brain injury; WD - Wilson disease.

The chelation therapy for non-metal overload indications continues to be investigated. Concerns remain that the most vulnerable infants are likely to acquire BIND, either because their exposure to bilirubin is not identified as severe enough to need treatment or is prolonged but slightly below current threshold levels for intervention.4,5 In acute neuronal insult events, such as stroke, traumatic brain or spinal cord injury and pathological processes of secondary neuronal injury play a key role in the severity of insult and clinical prognosis.6 Along with nitric oxide (NO) and carbon monoxide (CO), hydrogen sulfide (H2S) is regarded as the third gasotransmitter and endogenous neuromodulator and plays multiple roles in the central nervous system (CNS) under physiological and pathological states, especially in secondary neuronal injury. The mechanisms of secondary neuronal injury exacerbating the damage caused by the initial insult includes microcirculation failure, glutamate-mediated excitotoxicity, oxidative stress, inflammatory responses, neuronal apoptosis and calcium overload. The time has come to discover the differences and similarities in the actions of these gasotransmitters: NO, CO and H2S.7

Our observations suggest that D-PA has important neuroprotective effects in cases jeopardized by BIND or ROP. These unexpected effects may be related to D-PA
capability to alter the NO system.\textsuperscript{[8–12]} NO synthesized in the CNS produces a myriad of effects. For example, it plays a role in the control of blood flow, learning and memory, neurotransmitter release, gene expression, immune responsiveness, and cell survival. It is also implicated in numerous pathologies such as Alzheimer disease (AD), Huntington’s disease, and cerebral ischemia and disorders of the basal ganglia (BG) caused by metals (in Wilson disease /WD), bilirubin (in BIND) or other pathologic conditions (in Parkinsonism).\textsuperscript{[13]} D-PA is a hybrid drug in the neonatal period by its ability to modulate both oxidative stress and NO pathway. Tataranno et al.\textsuperscript{[14]} have summarized the new body of knowledge about antioxidant drugs for neonatal brain injury. D-PA-therapy of newborn infants may also have significant neuroprotective effects in cases jeopardized by BIND or ROP – (despite its peripheral location, the retina or neural portion of the eye, is actually part of CNS\textsuperscript{[15]}). These effects based on the capability of D-PA to alter the NO system and it is a strong antioxidant.\textsuperscript{[16, 17]} Low molecular weight disulfides are the major products of D-PA metabolism in humans.\textsuperscript{[18]} The oxidation of D-PA in vivo may also important in the mode of action of the drug through simultaneous reduction of the reactive oxygen species (ROS). So, we can say that 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.\textsuperscript{[19]} Moreover, D-PA irreversibly binds to primary aldehydes and scavanges peroxinitrite. In isolated rat brain mitochondria, this drug reduced peroxinitrite-induced mitochondrial respiratory failure, accompanied by a decrease in 4-Hydroxynonenal (4-HNE) level. In addition, D-PA administration in the acute phase of mouse traumatic brain injury (TBI) model aided recovery. The carbonyl scavenger D-PA binds primarily to aldehydes in an irreversible manner which inhibits their damaging effects and has also been shown to scavange peroxynitrite as well. Acute D-PA administration has previously been shown to improve neurological recovery in the mouse concussive head injury model and to protect brain mitochondria.\textsuperscript{[20, 21]} Furthermore, D-PA attenuates oxygen radical induces pulmonary hypertension of newborn pigs\textsuperscript{[22]} and probably prevents bronchopulmonary dysplasia (BPD) in premature babies. Other endogenously generated small-molecule species, such as CO and H\textsubscript{2}S, have also been shown to possess important signaling properties. These species play critical roles in numerous biological processes, including regulation of enzyme activity, protein structure and function and cellular defense.

**D-PA and CO production**

The gaseous neurotransmitter NO has already been shown to have an important regulatory function. However, little is known about the role of other neurotransmitters. Previous studies indicate that CO has a dual behavioral role within the anterior hypothalamus, exerting control over both reproductive and anxiety behaviors and that its similarities and contrasts to NO may stem from the variable regulation of the two CO producing enzymes: heme oxygenase-1 and -2 (HO-1 and HO-2).\textsuperscript{[23]} HO-1 is an inducible 32-kDa protein, while HO-2 is a constitutively synthesized 36-kDa protein and generally is unresponsive to any of the inducers of HO-1.\textsuperscript{[24]} The heme is an essential prosthetic group of enzymes with functions such as oxygen storage and transport (hemoglobin and myoglobin), electron transport and energy generation (NADPHoxidase, guanilylcyclase and cytochrome P-450 family); and enzymatic systems such as catalase, peroxidase, iNOS and cyclooxygenase. HO-1 induction leads to increased heme breakdown (e.g., hemolytic diseases and neonatal hyperbilirubinemia /NHBI), resulting in the production of iron, CO and biliverdin IX, which is subsequently reduced to bilirubin IX by biliverdin reductase. There is also evidence that HO-2 participates in a multitude of housekeeping functions, mainly in the brain, since it is the most prominent expressed isoform and the first to respond against oxidative stress. Indeed, the relevance of HO-2 for the CNS is emphasized by evidence showing that the continuous and regulated endogenous CO production by the activity of this enzyme is a key factor for maintaining the physiological function in neuronal cells and the vascular tone regulations of the cerebral blood vessels.\textsuperscript{[25]} It is also an interesting phenomenon that D-PA inhibits the rate limited enzyme (HO-1) in heme metabolism only in neonates\textsuperscript{[26]} and most likely, it does not have any effects on HO-2. As a part of age related effects this drug induces cytochrome P-450 in the neonatal period.\textsuperscript{[27]} The selective inhibition of HO-1 isoform is generally preferable.\textsuperscript{[28]} Because those enzymes that play an important role in antioxidant defense and drug metabolism are heme proteins, it can be assumed that in preventing hyperbilirubinemia, ROP and oxygen toxicity, the mechanism of action of D-PA is identical: the protection of biomembranes against lipid peroxidation caused by free radical.

**D-PA exerts inhibitory action on hydrogen sulfide biosynthesis**\textsuperscript{[29]}

Hydrogen sulfide (H\textsubscript{2}S) is regarded as the third gasotransmitter and endogenous neuromodulator and plays multiple roles in CNS under physiological and pathological states, especially in secondary neuronal injury.\textsuperscript{[30–31]} At high H\textsubscript{2}S content of CNS elevates neuronal Ca\textsuperscript{2+} concentration and may contribute to the formation of calcium overload in secondary neuronal injury. The above mentioned inhibitory effect of H\textsubscript{2}S can be also beneficial to develop of BIND or ROP.

**Copper is an endogenous modulator of neural circuit spontaneous activity**

Dodani et al.\textsuperscript{[32]} have shown that acute copper chelation in a dose-dependent manner in dissociated hippocampal culture and intact developing retina increased the cell participation and frequency of calcium transients during spontaneous activity. Consequently, the above mentioned
inhibitory effect of H₂S can be also beneficial to develop of BIND or ROP.

Moreover, modulation of cellular copper levels through genetic knock down of the copper ion channel copper transporter 1 (CTR1) led to a similar increase in synchronization of calcium transients, indicating that this protein is involved in dynamic regulation of copper signaling, which in turn affects neural activity. In addition, these data implicate Cu⁺ signaling in neuronal signaling, suggesting that alterations in brain copper homeostasis in genetic disorders like WD, as well as more complex neurodegenerative diseases such as AD and Huntington’s diseases and prion encephalopathies that are linked to copper mismanagement, can contribute to misregulation of cell-cell communication. D-PA is actually the drug most extensively used to treat copper overload in WD and as such is a very attractive building block for the design of chelating agents which is useful in neonates who have increased concentration of copper in their brain, particularly in the BG.⁷⁵

CONCLUSION

Our observations – together with other convincing cases participating in the long-term (28-40 years) follow-up – suggest that DPA-therapy in the neonatal period may have significant neuroprotective effects in cases jeopardized by BIND or ROP. According to our hypothesis D-PA can modulate the function of gasotransmitters and alters the copper homeostasis in the brain, so, it can protect the brain (especially the basal ganglia and retina) from various injury, such as BIND and ROP. During the last 40 years Hungarian neonatologists have treated a number of term and preterm infants with D-PA to treat severe jaundice and prevent ROP. No acute or long-term adverse effects or any late complications of this treatment protocol have been observed during several years of follow-up. According to our opinion, the most important „discovery” of D-PA-project is that this drug should be undoubtedly effective (jaundice, lead burden)⁷⁵ and may be in the prevention of ROP (a well-designed large multicenter randomized controlled trial is required) and vertical infection of HIV,⁷³ safe (more than 25-30000 cases only in Hungary without any side effects!) and quite inexpensive (even more for the developing countries!), and it can be used in unusually high doses as a short-term therapy in the neonatal brain’s defence.⁷⁶

REFERENCES


