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CHELATION THERAPY IN THE NEONATAL PERIOD: D-PENICILLAMINE CAN EXERT NEUROPROTECTIVE EFFECTS IN KERNICTERUS AND RETINOPATHY OF PREMATURITY

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ABSTRACT: This review covers of the results of previously conducted retrospective and prospective clinical trials and data of Cochrane reviews to examine the effects of D-penicillamine (DPA) for neonatal hyperbilirubinemia and associated low incidence of retinopathy of prematurity (ROP). In the ABO- and Rh-Hemolytic Disease of the Newborn (HDN) DPA significantly reduced the need for both initial and repeated exchange transfusions (ET). In Rh-HDN, almost the half of cases, no ET was performed in the DPA-treated group. Furthermore, this treatment was associated with elimination of all stages of ROP in two trials conducted between 1984 and 1986 in the Department of Pediatrics, Medical University of Debrecen. DPA-therapy of newborn infants may have significant neuroprotective effects in cases jeopardized by bilirubin-induced neurologic dysfunction (BIND) or ROP, which may be related to its capability to alter the nitric oxide (NO) system and to its strong antioxidant effects. It can be assumed that in preventing and treating hyperbilirubinemia, ROP and oxygen toxicity, the mechanism of action of DPA is identical: the protection of biomembranes against lipid peroxidation caused by free oxygen radical. Conclusion: It is important to note that there was no intolerance or short- or long-term toxicity of the medication, in spite of the fact that in the newborn period DPA was used 10-20 times higher doses than those in adult.

INTRODUCTION: Chelation therapy is routinely performed for cases of heavy metal overload in the clinical practice. The use of chelation therapy for non-overload indications continues to be investigated. This review addresses the medical necessity of chelation therapy in the neonatal period. The idea that DPA might be a suitable drug to act as a copper-binding agent for use to control icterus neonatorum occurred, serendipituously, to one of us (L.L.), while reflecting on the similarity of copper storage in Wilson's disease and neonates [1, 2].

It is well known that all neonates have increased concentration of copper in their liver and in their brain (particularly in the basal ganglia), and a decreased concentration of a specific plasma copper-protein, coeruloplasmin, in comparison with individuals over one year old.

D-Penicillamine:

[β, β-dimethyl-D-cysteine] **Fig.1**: Shows the DPA molecule which was discovered among the hydrolysis products of penicillin by Abraham *et al.* In 1942 [3].

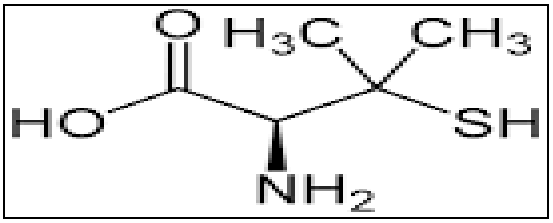

FIG.1: STRUCTURAL FORMULA OF D-PENICILLAMINE

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Dosages and use of D-Penicillamine in neonates: 3 x 100 mg/kg bw./day intravenously or orally for 3-7 days in the neonatal jaundice + once daily 50 mg/kg bw. intravenously until the end of the second week of life to prevent retinopathy of prematurity (ROP).

Clinical observations in the treatment of neonatal hyperbilirubinemia (NHBI): Table 1 shows the effects of DPA-therapy in ABO- and Rh-Hemolytic Disease of the Newborn (HDN) in term infants (IV administration starting at <24 hours of age). In the ABO- and Rh-HDN, DPA significantly reduced the need for both initial and repeated exchange transfusions (ET). Infants who received DPA therapy had significantly lower mean serum bilirubin (SEBI) concentrations than the control infants [4-6].

TABLE 1: EFFECTS OF D-PENICILLAMINE IN ABO- AND Rh-HAEMOLYTIC DISEASE OF THE NEWBORN INFANTS.

	ABO-HDN		Rh-HDN	
	DPA-group	**Controls**	**DPA-group**	**Controls**
N (M:F)	34 (15:19)	34 (12:22)	30 (18:12)	33 (19:14)
Cord bilirubin (mg/dL)	3.9	3.9	3.9	4.2
Serum bilirubin <24 hs	11.1	11.9	11.2	12.3
Peak bilirubin at 48-72 hs	15.0	18.4	14.0	15.6
Exchange transfusions	3 (X:0.11)	25 (X:1.3)	21 (X:0.7)	52 (X:1.6)
ETs were not performed			43.3 %	6 %

N (M:F) = number (male: female); X = mean number of ETs/newborn baby

In the course of conducting clinical trials to investigate the presumably beneficial effects of DPA in the reduction of ROP, we routinely measured the SEBI of VLBW infants. There was no significant difference between the DPA-treated and control groups either in the mean peak SEBI or in the number of ETs needed (**Table 2**) [7].

TABLE 2: EFFECT OF PHOTOTHERAPY (PhT) AND DPA VS. PhT IN INFANTS <1500 g bw.

	PhT + DPA	**PhT**
N (M:F)	**25 (12:13)**	**23 (12:11)**
Serum bilirubin (mg/dL)	9.8	
Before treatments	12.2	10.0
Peak bilirubin at 5-6 days	6	12.8
ETs		6

Recently we published case reports including those most serious, extremely hyperbilirubinemic infants who recovered without any neurologic dysfunction after DPA therapy [8]. These cases are all the more remarkable as the most common sequelae of neonatal hyperbilirubinemia is the sensorineural hearing impairment [9].

D-penicillamine a non-bilirubin displacing drug in the neonatal period were performed detailed investigations using three *in vitro* methods (Sephadex method, peroxidase technique, MADDS – monoacetyldiamino-diphenylsulfone – method) in addition to two *in vivo* testing in Gunn rats [10]. Results were negative in all cases. Quantitatively, the doses of DPA administered to the neonates do not displace bilirubin from its binding to albumin.

Mechanisms of action of D-penicillamine in the NHBI:

Three crucial areas of bilirubin formation and excretion have been investigated in our laboratory: (1) the lipid peroxidation of the red blood cell membrane and hemolysis [11], (2) heme oxygenase-, and (3) UDP-glucuronyltransferase enzyme activity, before and after DPA treatment.

➤ The susceptibility of red cell lipids to autooxidation is about three times as high in the newborn as in adults [12]. *In vitro*, the preincubation with DPA resulted in a significant decrease of both the hemolysis and fluorescence of red cell lipid extracts [13]. *In vivo*, pretreatment with DPA has prevented the phenylhydrazine-induced lipid peroxidation in rats. The binding of DPA to malondialdehyde may prevent this process [14, 15].

➢ After this we examined the activity of heme oxygenase, the initial and rate-limiting enzyme of heme degradation [16]. The 3 days of DPA treatment in the adult animals did not lead to any significant change; in contrast, in neonates a marked reduction in enzyme activity was observed following DPA treatment [17].

➢ After DPA treatment we could not observe any changes in enzyme activity of UDP-glucuronyltransferase [18].The plausible explanations of age-relating mechanisms of action of DPA are as follows: bilirubin production will be inhibited by the decreased activity of heme oxygenase which is supported by the experimental works of Maines and Kappas [19]. The high activity of heme oxygenase in the newborn could reflect the enzyme-inducing action of metals derived from the breakdown of fetal erythrocytes [20]. Chelation therapy in neonates restores the normal (adult) enzyme activity.

Prevention of ROP with DPA (clinical observations and randomized conrolled trials):

Improved survival of low birth weight, premature babies has led to increased levels of disability and associated defects, including ROP-incidence, mainly among the "fetal infants - micropreemie" that survive with birth weight about 500 g and 22-25 weeks of gestation [21]. The history of DPA therapy in neonates under 1500g birth-weight can be divided into four periods. During the first period we used DPA only against neonatal jaundice. We then decided that all infants weighing less than 1500g birth weight and requiring supplemental oxygen should receive DPA therapy [22, 23].

During the second and third period the new mode of DPA-administration was still not able to totally eradicate the occurence of RLF (**Table 3**). During the fourth period we conducted a strictly controlled prospective trial to investigate the presumably beneficial effects of DPA not only in the prevention of the cicatricial form of the disease but also in the reduction of the acute stages [7]. Summarizing the results of two controlled randomized prospective trials carried out at different times, it can be seen that both trials included infants who had birth weights <1500g. 270 preterm babies of 26 to 33 weeks gestational age were enrolled in the study.

TABLE 3: HISTORY OF D-PENICILLAMINE TREATMENT OF NEONATES < 1500 g BIRTH WEIGHT

	First period (1973 – 1979)	Second period (1979 – 1980)	Third period (1981 – 1982)
Dosage and administration	**300 mg/kg bw. IV for 3 days**	**300 mg/kg bw. IV for 3 days**	**300 mg/kg bw. IV for 3 days + 50 mg/kg IV for 2 weeks**
Number of survivals	193	133	152
DPA-treated	61	133	152
Retrolental fibroplasia	1	1	1
Untreated	132		
Retrolental fibroplasia	10		

TABLE 4: INCIDENCE OF ROP IN THE STUDY POPULATION

		Staging of the disease			Total ROP (%)
	Normal	**I**	**II**	**III**	**IV**
DPA (*n*)	70				0
Control (*n*)	53				9 (14.5)
751-1000 g	1		1		1
1001-1250	17	1	2	1	2
1251-1500	35				

79 died before 10 weeks of age and were not evaluated for the presence of ROP. The high mortality rate could be explained by the facts that nearly 30 years ago we had to work in unfavorable circumstances: outpatient babies transferred by conventional ambulance, no surfactant therapy, old- fashioned equipment et cet. 132 babies completed the trial: 70in the DPA- and 62in the control group. During the 22-month study period nine infants were diagnosed as having ROP stage I or greater during their hospital stay. Both eyes were affected equally. All of these premature infants

belonged to the control group, so that, with respect to the frequency of the active phase of this disease, the difference between the DPA-treated and control group is statistically significant (**Table 4)**. Infants with ROP had gestational ages ranging from 27 to 31 weeks.

How Does D-penicillamine Work Against Retinopathy of Prematurity?

There is a wide agreement that the development of ROP is triggered by a number of conditions which can seriously disturb the retinal circulation resulting in ischemic retinopathy with the consequence of vasoproliferation and cicatrisation [24-26]. Of these factors (1) immaturity, (2) oxygen toxicity (which is not equivalent to supplemental oxygen therapy) and (3) neovascularisation are considered to be most important.

Maturation:

ROP is a pathologic process that occurs only in immature retinal tissue and can progress to a tractional retinal detachment which can result in functional or complete blindness [27-31].

Age-related effects of D-penicillamine:

Paediatric patients display different pharmacokinetic and pharmacodynamic responses to drugs. This is why we can speak about developmental or age-related pharmacology [32]. We demonstrate the results of our animal experiments regarding the age-related differences in effects of DPA in the **Table 5** [33].

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, i.e. DPA boosts or inhibits the immature enzyme systems to the adult level [34].

TABLE 5: 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
Radioprotection	significant	?

Oxygen toxicity:

Antioxidant effects of D-Penicillamine:

Low molecular weight disulfides are the major products of DPA metabolism in humans. The oxidation of DPA in vivo may also important in the mode of action of the drug through simultaneous reduction of oxygen species [35-38].

Neovascularization:

The pathophysiology of ROP understood to start with injury to the incomplete developing retinal capillaries. Once the developing vessels have been damaged, it is hypothesized that the retina responds with the production of VEGF stimulating neovascularization (which is the observable retinopathy) which may progress to neovascular membranes in the vitreous and subsequent scarring (cicatrix) and retinal detachment [39]. VEGF and its receptors are overexpressed in many tissues with blood vessel growth, often together with other angiogenesis factors. Recent research suggests that VEGF is one of the most important growth factor involved in the pathological mechanism of ROP and diabetic retinopathy [40].

Splitting of disulfide bridges by D-Penicillamine:

One of the oldest and well-documented effects of DPA is the splitting of intramolecular or intermolecular disulfide bridges. Through the control of peptide-disulfide regioisomer formation DPA can alter the biological profile of VEGF by providing a local constraint or cleavage on the adjacent disulfide bond as well as on the global peptide conformation [41].

Copper and the vasculogenesis: Copper was shown to stimulate blood vessel formation in the

avascular cornea of rabbits. Cu privation by diet or by Cu chelators diminishes a tumor's ability to mount an angiogenic response. These data have shed new light on the functional role of Cu in microvessel circulation [42]. DPA was first use as a hevay metal chelator especially binding copper by its NH_2 group **(Fig.1).** To sum up and over-simplify the mechanisms of action of DPA to prevent ROP can be seen in the **Fig.2**.

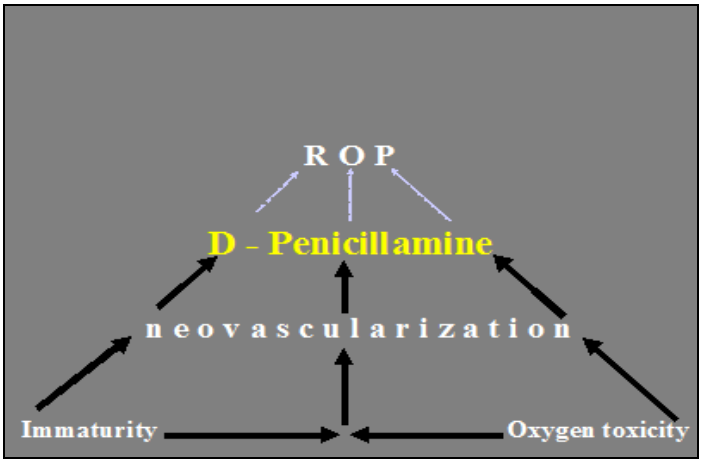

FIG.2: PROPOSAL MECHANISMS OF ACTION OF D- PENICILLAMINE IN THE PREVENTION OF RETINOPATHY OF PREMATURITY

DPA is a hybrid drug in the neonatal period by its ability to modulate both oxidative stress and nitric oxide (NO) pathway:

Tataranno *et al* [43] have summarized the new body of knowledge about antioxidant drugs for neonatal brain injury. Our recently published case reports [8] together with other convincing cases participated in the long-term (28-40 years) follow-up – suggested that DPA-therapy of newborn infants may have significant neuroprotective effects in cases jeopardized by bilirubin-induced neurologic dysfunction (BIND) or ROP (→despite its peripheral location, the retina or neural portion of the eye, is actually part of central nervous system [44]**)**. This unexpected effect may be related to DPA capability to alter the nitric oxide (NO) system [45-48], and its strong antioxidant effects [49-51].

NO synthesized in the central nervous system 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's disease, Huntington's disease, and cerebral ischemia, and disorders of the basal ganglia caused by metals (Wilson's disease), bilirubin (BIND) or other pathologic conditions (Parkinsonism). The use of chelation therapy for non-metal overload indications continues to be investigated. Furthermore, the mechanism of DPA in the reduction of serum bilirubin based on the fact that this drug inhibits the rate limited enzyme (heme oxigenase) in heme metabolism [17]. 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 hyperbilirubinemia, ROP and oxygen toxicity, the mechanism of action of DPA is identical: the protection of biomembranes against lipid peroxidation caused by free radical. Low molecular weight disulfides are the major products of DPA metabolism in humans [35].

The oxidation of DPA in vivo may also important in the mode of action of the drug through simultaneous reduction of oxygen species. Finally, we can say that DPA 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 [52].

Moreover, DPA irreversibly binds to primery aldehydes and scavanges peroxinitrite. In isolated rat brain mitochondria, DPA reduced peroxinitrite-induced mitochondrial respiratory failure,

accompanied by a decrese in 4-Hydroxynonenal (4-HNE) level. In addition, administration this drug in the acute phase of mouse traumatic brain injury (TBI) model aided recovery. So, it acts as carbonyl scavanger and is neuroprotective in TBI models [53]. We think that this raises a number of important questions, as it seems to be a real problem. These are not mere details or points to be left for another day [54].

Safety and tolerance of D-Penicillamine:

Wilson's disease patients and presumably the neonates are relatively protected against adverse effects because the great excess of copper may block the –SH group in the free DPA from forming such a haptenic antigen. Furthermore, we must stress that there are no immunosuppressive effects of this drug in neonatal period, particularly in the course of a short-term therapy [55].

Results of 1-year follow-up revealed no difference between the two groups in respect to somatic growth, development and neurological outcome. At the same time the DPA-treated group showed a significant adventage over controls in regards rehospitalization and ophtalmological outcome including ROP and other visual impairments [56-58].

Non-replication of the replicable:

Dr. William A. Silverman has written the above quoted title in his book-chapter [59]. We can say that until Silverman's „declaration" only sporodical publications appeared in Hungarian and Polish journals [60-64], and later in Mexico [65], mainly about the treatment of neonatal jaundice. Then, we published a provocative letter [66] to persuade others to perform randomized controlled trials in the prevention of ROP.

The publications of Christensen *et al.* [67] can be considered as the first international replications of our observation and clinical trials. They also recognised no immediate intolerance of the prepared solution of DPA given by nasogastric tube, nor did they observe any evidence of renal, haematological, or hepatic toxicity in patients approved by the FDA. Recently, a research group in India has conducted a prospective controlled trial [68] without any reduction in the number of ROP in the DPA-treated group. This controversal outcome was reflected in the Cochrane rewiews [69, 70], as well. The explanation of differences lies (1) in the dosage of DPA (parenteral- or oral-treatment); (2) in the start of administration – within 12 hours or 3-5 days of age –, and (3) may be the genetic differences in susceptibility to advanced ROP [26]. It is a good thing and cleare-cut, however, that DPA was well tolerated and did not have any major short-term adverse effects.

CONCLUSIONS: During the last 40 years Hungarian neonatologists have treated approximately a number of term and preterm infants with DPA to treat severe jaundice and prevent retinopathy. 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 DPA-project is that this drug should be undoubtedly effective (jaundice, ROP, lead burden [71], pulmonary hypertension [45] or HIV (!) vertical infection [72] in neonates), safe (more than 25-30 000 cases only in Hungary without any side effects!) and quite inexpensive (even more for the developing countries!), and it can be used in unusual high doses in the neonatal period [73]. So, the risk vs. benefit ratio of DPA-treatment – as is to be expected [74] – is very low in the treatment of newborn infants

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