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CHELATION THERAPY IN THE NEONATAL PERIOD: D-PENICILLAMINE HAS POSSIBLE BENEFICIAL EFFECTS ON THE LETHALITY OF HIV OR EBOLA INFECTION DUE TO VERTICAL TRANSMISSION

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ABSTRACT: *D-penicillamine (DPA) was first recognized as a potential benefit for neonatal hyperbilirubinemia (NHBI). During this time there was a remarkedly low incidence of retinopathy of prematurity (ROP) in the infants treated with DPA. Later, our studies were replicated in other institutes in Hungary, Poland, U.S. A., India and Mexico. 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. On the basis of an American research work concerning the beneficial effects of DPA-therapy in adult AIDS-patients (although in these cases there were many unpleasant, adverse effects), it would be reasonable to treat neonatal HIV- or Ebola-positivity due to vertical transmission with short-term DPA therapy (300 - 400 mg/kg/bw/day for 5-7 days). In addition, neonates born to mothers with Ebola virus disease have not survived yet, i.e. the lethality of this disease is 100 %. Therefore, we have a moral obligation to help the fight against HIV and EBOLA with this inexpensive (~30 US Dollar/baby) drug in the neonatal period.*

KEYWORDS: D-Penicillamine, Neonatal Hypeerbilirubinemia, Retinopathy of Prematurity, Hiv, Aids, Ebola, Therapy.

INTRODUCTION

When in the early 1970s, we reviewed the role of D-Penicillamine (DPA) in the treatment of NHBI [1], the drug was new to most neonatologists. 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 [2]. It is well known that all neonates have increased concentration of copper in their liver and a decreased concentration of a specific plasma copper-protein, ceruloplasmine, in comparison with individuals over one year old.

In this survey we review our DPA research, which embraces a period of more than 40 years. We intend to focus only on a few aspects of this field which we guess to be most important.

D-Penicillamine

Figure 1 shows the DPA molecule which was discovered among the hydrolysis products of penicillin by Abraham et al. in 1942 [3]. It is now more than 50 years since Walshe [4] introduced DPA into the treatment of Wilson's disease. Then, it was established as the treatment of choice for cystinuria [5, 6]. Subsequently, this drug found application in various forms of heavy metal intoxication [7, 8] and, its clinical use in rheumatoid arthritis was accepted as well [9]. Bizarrely, DPA is a very cheap, low-cost drug, but at the same time it is developed under the Orphan Drug Act of 1983 in the U.S. which is a federal law concerning rare

diseases (orphan diseses) [10]. This means that the pharmaceutical companies produce this „homeless, not a money-maker" drug with reluctance. For example the IV form of DPA is nowdays not available in the market and the per os preparation is produced by a few companies in the world. Dosages and the use of DPA in neonates can be seen in the **BOX 1**.

Clinical observations in the treatment of NHBI

Evidence from randomized trials is essential to inform rational choices made by health care providers [11, 12, 13]. In the early 1970s, however, there were too many seriously jaundiced babies cared for in the neonatal departments in Hungary. So, we used a „special Hungarian randomization" at that time: babies (controls) constituted the comparison group for studies who were cared for in the same neonatal department when the gift (Metalcaptase® - from Knoll AG Ludwigshafen, West-Germany) did not reach us, ie. it was not available. **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-HDN, DPA significantly reduced the need for both initial and repeated exchange transfusions (ET). The number of ET per infant was 1.32 in the control and 0.11 in the treated group. The infants who received DPA therapy had significantly lower mean serum bilirubin (SEBI) concentrations than the control infants [14]. In Rh-HDN the number of ET per infant was 1.6 in the control and 0.7 in the treated group. In addition almost the half of cases no ET was performed in the DPA-treated group [15]. In another clinical study[16] we examined the ability of DPA therapy to modify the course and duration of so-called idiopathic hyperbilirubinemia in term infants in comparison with ET (**Table 2**). Patients were randomly selected to receive DPA therapy or ET when the SEBI reached values of more than 20 mg/dL. It is to be noted that ET was also performed in cases treated with DPA where the level of bile pigment did not decrease within 4-6 hours after the first single intravenous dose. No infants studied had any laboratory or clinical evidence of illness or hemolytic process or any sings of disturbances of the central nervous system. It was found that infants who received ET had a significantly lower SEBI 8-12 hours after intervention than infants in the DPA-treated group, but there was no significant difference between the two groups at 32-36 hours of the postexchange period, respectively.

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 [58]. This suggests phototherapy alone proved to be just as effective as phototherapy plus DPA in babies with a birth weight under 1500 g **(Table 3)**.

Case reports

There were some very impressive cases in our practice in neonatology which deserved to be shown individually.

The first patient received DPA treatment in the neonatal period was an AB0-incompatible preterm infant. At an extremely high SEBI (32.5 mg/dL), and signs of various neurological dysfunction, intravenous administration of DPA was started. The first dose caused a spectacular fall of 6.5 mg/dL in the level in 4 hours and, under the influence of such treatment, we were able to witness a gradual disappearance of the NHBI. She is now a member of a famous operhouse in Germany as an opera singer [17]. This case is all the more

remarkable as the most common sequelae of NHBI is the sensorineural hearing impairment [18]. She and her daughther of eighteen can be seen in **Figure 2.**

In 1999 we published a case of an AB0 incompatible term infant girl born to parents who were Jehovah's Witnesses [19]. The infant was admitted to our neonatal unit with high SEBI necessitating ET, but her physical and neurological status was good. The parents signed a request that blood should not be administered under any circumstances. However, they authorised the use of alternative treatments: orally administered DPA, phototherapy, intravenous fluids, and recombinant human erythropoietin (200 U/kg subcutaneously on every second day for two weeks). This infant was discharged from our unit in good health. Her physical growth and motor milestones at 3 years of age revealed no red flags for neurodevelopmental maturation. In addition, the follow up audiometric tests performed on this infant were normal. She was the first baby in the world who received such a combined alternative (and "bloodless") treatment for serious AB0-HDN.

We recently cared for a term infant boy blood group B, Rh-positive who was born at 37. weeks of gestation to a 33-year old, blood group B, Rh-negative mother [20, 21]. The baby was born as an 11th offspring of his mother and appeared jaundice at 10 hours of life and had moderate anaemia. No sign of neurological dysfunction. The direct Coombs test was strongly positive (++++) in the cord blood. The clinical characteristics of the infant with Rh-HDN are shown in the **Box 2.**

D-penicillamine a non-bilirubin displacing drug in the neonatal period

It is appropriate to elucidate drug's interference with the binding of bilirubin to human serum albumin. We 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 [22, 23, 24]. 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 neonatal hyperbilirubinemia

The complete mechanism of action of DPA is still unknown, but some interesting pieces of information have been unfolded over the last decades. Three crucial areas of bilirubin formation and excretion have been investigated in our laboratory: the lipid peroxidation of the red blood cell membrane and hemolysis; heme oxygenase –, and UDP-glucuronyltransferase activity, before and after DPA treatment. Lipid peroxidation has been considered to be a mechanism of membrane damage in a number of red cell disorders leading to hemolysis [25]. The susceptibility of red cell lipids to autooxidation is about three times as high in the newborn as in adults [26]. In vitro, the preincubation with DPA resulted in a significant decrease of both the hemolysis and fluorescence of red cell lipid extracts [27].

In vivo, pretreatment with DPA has prevented the phenylhydrazine-induced lipid peroxidation in rats [28]. Malondialdehyde is a product of lipid peroxidation resulting in disintegration and disruption of biologic membranes [29, 30, 31]. The binding of DPA to malondialdehyde may prevent this process [32, 33, 34].

Since heme metabolism is a crucial stage in bilirubin production, we examined the activity of heme oxygenase, the initial and rate-limiting enzyme of heme degradation [35, 36]. The 3 days of DPA treatment in the adult animals did not lead to any significant change in heme oxygenase

activity. In contrast, in neonates a marked reduction in enzyme activity was observed following DPA treatment. At the same time, the activity of UDP glucuronyltransferase was measured in liver homogenates of newborn and adult rats. After DPA treatment we could not observe any changes in enzyme activity.

The plausible explanation of age-relating mechanisms of action of DPA: bilirubin production will be inhibited by the decreased activity of heme oxygenase. The age-related differences in the effect of DPA concerning heme oxygenase is supported by the experimental works of Maines and Kappas [37]. The high activity of heme oxygenase in the newborn could reflect the enzyme-inducing action of metals derived from the breakdown of fetal erythrocytes [38, 39]. Chelation therapy in neonates restores the normal activity of enzymes participating in heme metabolism.

Thus, chelating agents facilitate heme synthesis and inhibit heme degradation [40, 41, 42, 43].

In the light of the foregoing we present our clinical observations in **Figure 3.** The effect of a single 100 mg/kg body weight intravenous dose of DPA on SEBI in premature and term infants can be seen after 4-6 hours of the administration. A rapid decrease in SEBI was observed only in term infants with high SEBI, but DPA has not had any effect in prematures under 1500 g birth weight (the WLBW infants suffering from so called accumulating NHBI due to immaturity of glucuronyltransferase enzyme system) and term infants with low SEBI. A plausible explanation for this is that DPA inhibits bilirubin formation but it does not cause any change in UDP-glucuronyltransferase activity. In cases with high bilirubin, however, the marked decrease observed was due to enzyme induction by bilirubin itself, which had gradually increased during the previous days in these babies [16].

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

Improved survival of low birth weight, premature babies in developing countries has increased the incidence of ROP [44, 45]. Regrettedly, this increased survival has also led to increased levels of disability and associated defects mainly among the so called "fetal infants - micropreemie" that survive with birth weight about 500 g and 22-25 weeks of gestation [46]. According to a recent World Health Organization report [47], ROP is emerging as a maior cause of blindness in childhood. The disease prevention seems to be especially important because the therapy of ROP cases with cryotherapy or current methods of treatment rely on highly invasive laser procedures [48] that themselves lead to some vision loss. The biggest drawback of anti-VEGF (vascular endothelial growth factors) treatment with intravitreal Bevacizumab/Avastin® is exposing an immature infant to a drug for which experts cannot evaluate the systemic risk. For example, these infants with ROP are already prone to bronchopulmonary dysplasia, and the most significant target organs for damage from VEGF supression are the alveoli. [49, 50].

Pathogenesis of ROP.

Many maternal, fetal, environmental and nutritional factors have been implicated in the development of this condition, but whether each is independently significant in ROP causation or simply an associated factor indicative of an immature and ill neonate has in many instances yet to be determined. So, ROP is to be consider as paradigma of a multifactorial disease of the developing retinal circulation [51, 52].

The history of DPA therapy in neonates under 1500 g birth-weight

Can be divided into four periods. During the first period we used DPA only against neonatal jaundice. The original aim of our retrospective screening program, carried out in 1979, was to estimate the incidence of retrolental fibroplasia (RLF) [53]. It was surprising that among DPA recepient babies there was only one case of RLF, whereas ten out of the 132 without such treatment developed severe cicatricial stages of the disease. We then decided that all infants weighing less than 1500 g birth weight and requiring supplemental oxygen should receive DPA therapy [54]. During the second period of DPA treatment there was one case out of 133 infant survivals where RLF developed. This baby received three days DPA treatment and three weeks oxygen therapy. We then changed the dosage and duration of DPA administration. During the third period the new mode of DPA-administration was still not able to totally eradicate the occurence of RLF (**Table 4.**).

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 [55].

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 <1500 g.

270 preterm babies of 26 to 33 weeks gestational age were enrolled in the study.

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: 70 in the

DPA group and 62 in 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 5.)** [56, 57, 58, 59, 60, 61]. Infants with ROP had gestational ages ranging from 27 to 31 weeks.

Conclusions: *First,* DPA treatment was associated with elimination of all stages of ROP in this trial. *Second,* in this randomized, single-centered comparison analysis, a 14-day course of DPA resulted in no apparent short- and long-term toxicity [62, 63, 64, 65].

How Does D-penicillamine Work Against Retinopathy of Prematurity?

The etiology of ROP is now accepted as multifactorial. 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 cicatrization [66].

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

during fetal life and after birth is a process involving all organs and functions of the growing human. *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 [67, 68].

Age-related effects of D-penicillamine in the neonatal period

Paediatric patients display different pharmacokinetic and pharmacodynamic responses to drugs. This is why we can speak about developmental or age-related pharmacology [69]. In the **Table 6.** we demonstrate the results of our animal experiments regarding the age-related differences in effects of DPA [70]. 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 level [71].

Oxygen toxicity

The discovery of free radicals, led to an enhanced understanding of endogenous enzymatic and nonenzymatic antioxidants that have evolved to counter the adverse effects of endogenous reactive oxygen species. 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 and oxygen toxicity, the mechanism of action of DPA is identical: The protection of biomembranes against lipid peroxidation[72, 73, 74]. A series of conditions in neonates may, at least partly, be caused by oxygen radicals, e.g. bronchopulmonary dysplasia, ROP, necrotising enterocolitis, patent ductus arteriosus and may be bilirubin encephalopathy [75].

DPA as an antioxidant drug in the neonatal period

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 (**Box 4.)** [76, 77, 78, 79, 80, 81].

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 [82]. 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 [83, 84, 85].

Splitting of disulfide bridges by DPA

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

(**Box 5.)** [86, 87, 88, 89, 90].

Copper and the vasculogenesis

Although two decades have passed since copper was shown to stimulate blood vessel formation in the avascular cornea of rabbits, only recently have clinical trials established that

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 development (**Box 6.**) [91].

DPA as a haevy metal chelator

DPA was first use as a hevay metal chelator especially binding copper by its NH_2 group **(Figure 1.).**

To sum up and over-simplify the mechanisms of action of DPA to prevent ROP can be seen in the **Figure 4**.

Safety and tolerance of D-Penicillamine or else **„nomen est omen"**

The name of this drug has become frightening for doctors and, especially, for neonatalogists, because long-term administration with the protocol of „go low – go slow" in rheumatoid arthritis *(the motto of neonatal administration is „go high – for a while")* patients resulted in more than 20% advers effects [92, 93].

In rheumathoid arthritis there is a background of disturbed immune system in contrast in patients with Wilson's disease and neonates with Wilson's disease-like condition [94]. So one can conceive that in RA an haptenic antigen may form in which the haptene is the DPA, which has combined with a variety of proteins and paraproteins, as well.

Wilson's disease patients and presumably the neonates are relatively protected because the great excess of copper may block the –SH group in the free DPA from forming such an antigen [92]. 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 [95, 96, 97, 98]. It is also noteworthy that there is a significant age-related difference in the acute toxicity of DPA administered parenterally to the animals (**Box 7, 8.**) [99].

The success of neonatal intensiv care can be judged by two crucial aspects (**Box 9.**) [100]. We have numerous theoretical and practical evidence for the lack of side effects in the neonatal period. 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 [101, 102, 103].

Non-replication of the replicable

Dr. William A. Silverman has written the above quoted title in his book-chapter [104]. He was the father of neonatal intensive care, and the author of a far-famed book [105]. Bill proved to be our greatest supporter [106, 107] and he reviewed our reseach work in details, and insisted on the importance of replications. We can say that until Silverman's „declaration" only sporodical publications appeared in Hungarian and Polish journals [108, 109, 110, 111, 112], mainly about the treatment of neonatal jaundice. This fact was especially distressing for us because in the 1970s and 1980s years the DPA therapy was widely used in Hungarian hospitals for

preventing ETs in the treatment of NHBI[113]. As far as we know DPA therapy was recently tried in Mexico [114] in the neonatal icterus, as well. Then, we published a provocative letter [115] to persuade others to perform randomized controlled trials in the prevention of ROP.

The above mentioned letter and a personal meeting in Utah resulted in publications of Christensen and his coworkers [116, 117] which 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. Later, a research group in India conducted a prospective controlled trial [118] without any reduction in the number of ROP in the DPA-treated group. This controversal outcome was reflected in the Cochrane rewiews concerning the prevention of ROP [119, 120]. The explanation of difference lies (1) in the dosage of DPA (parenteral- or oral-treatment) and (2) in the start of administration (within 12 hours or 3-5 days of age). It is a good thing and cleare-cut, however, that DPA was well tolerated and does not have any major short-term adverse effects. Furthermore, this drug is a potentially useful agent not only in NHBI and in the prevention of ROP, but in the neonatal lead burden [121], in the persistent pulmonary hypertension of newborn [122, 123] and probably in the treatment of HIV positivity due to vertical transmission [124, 125]. So, this is an ideal drug for the realization of a win/win approach [126].

International replication, and the need for long-tem follow-up studies

Our results suggest that DPA administration in very low birthweight infants has no serious adverse effects during the neonatal period, nor during the short-term and (semi)long-term (10–11 years) follow up [127, 128]. Then, we decided to conduct a long term follow up study to survey the quality-of-life of two cohorts of adults (28-40 years of age) treated with DPA in the neonatal period to reduce their bilirubin level (term infants) or to prevent ROP (VLBW babies) at our department. This survey was carried out by mailing the EuroQol-5D questionnaire to the above cohorts. The mailing list contained 277 addresses. The EQ-5D [128] includes a descriptive profile and a single index value for health status. The visual analog scale (VAS) records the respondents' self-rating for their current HRQOL on a graduated (0-100) scale, with higher scores for higher HRQOL [129, 130]. The descriptive system is composed of 5 dimensions of health: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. A questionnaire was used to assess a health-behavior, current chronic health conditions and socio-economic status. Our data were compared to the results of a survey carried out on a representative sample of the Hungarian population in 2003 by Szende and Németh [129]. The comparison was age-matched. *Results*: respondent rate was 52,3 %. Of the 145 questionnaire 135 was appropriate for analysis, the rest was incomplete. The study sample consists of 119 premature and 16 term neonates. 64 of the total were male. No statistically significant difference was found neither in the EQ-5D health index total score nor in the VAS score between the study population and the normative scores of the Hungarian population. EQ-5D index score was 98.6 and 87.2 (term and preterm sample, resp.) versus 93.7 in the general Hungarian population. Similar result were found using the Visual Analoge Scale (0-100): 89.9 and 83.8 (term- and preterm- groups, resp.) versus 83.4 in the general Hungarian population. Adults born preterm reported more health problems in all EQ-5D dimensions than the matched general Hungarian population in type and frequency as well [131, 132]. Prevalence of chronic health conditions did not deviate from predictable rate. The data of

this pilot study is not sufficient for counting statistical significancy. Further data analysis of 377 respondents are in progress [133].

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 and lead burden 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. So, the risk vs. benefit ratio of DPA-treatment – as is to be expected [134] – is very low in the infection of HIV- or EBOLA virus, as well.

Possible beneficial effects of DPA on the lethality of HIV infection due to vertical transmission

West Africa is currently in the midst of the largest Ebola outbreak in history and HIV prevalence in sub saharan Africa is also very high. Therapeutic interventions targeted at influencing endothelial activation in newborn babies early during the course of infection might include drugs affect endothelial activation, such as DPA [135].

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 proteinsynthesis [136]. Searching the pertinent literature, several publications relating to the beneficial effects of DPA-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 DPA completely inhibited HIV replication in H9 cells in vitro [137], ***(a single 100 mg/kg bw. IV administered DPA resulted in more multiple plasma concentration in premature infants)*** [138]. Recently, Dr. Gorbee Logan, a Liberian physician, tried using lamivudine against Ebola out of sheer desperation. Only two out of 15 patients taking it died – far lower than the average death rate (~ 70%). Logan read about the medication and similarities between Ebola and HIV in a medical journal [139, 140]. So, this study has another 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, DPA-therapy may be a potent early regime to control HIV (or EBOLA) replication and offers the golden opportunity to prevent or reverse the rapid progression of these diseases. The potential mechanism of antiretroviral actions of DPA in infections caused by vertical transmission are as follows:

- It is presumed that antioxidant treatment (DPA is a wellknown strong 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 [141]

- It acts as a potent protease inhibitor in animal model [142].

- The copper metabolism in Wilson's disease and innewborn infants is strikingly similar: they both have largequantities 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 [143].

- Extra cystein given in the form of DPA (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 theintracellular transport of Zn and Cu ions [144].

- 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 HIV- or EBOLA- replication could be inhibited by this drug [145, 146, 147, 148, 150].

It would be very exciting to be involved in this work, especially since a pilot study (5 babies) could be enough to prove that DPA will have a huge impact on HIV or EBOLA infection caused by vertical transmission.

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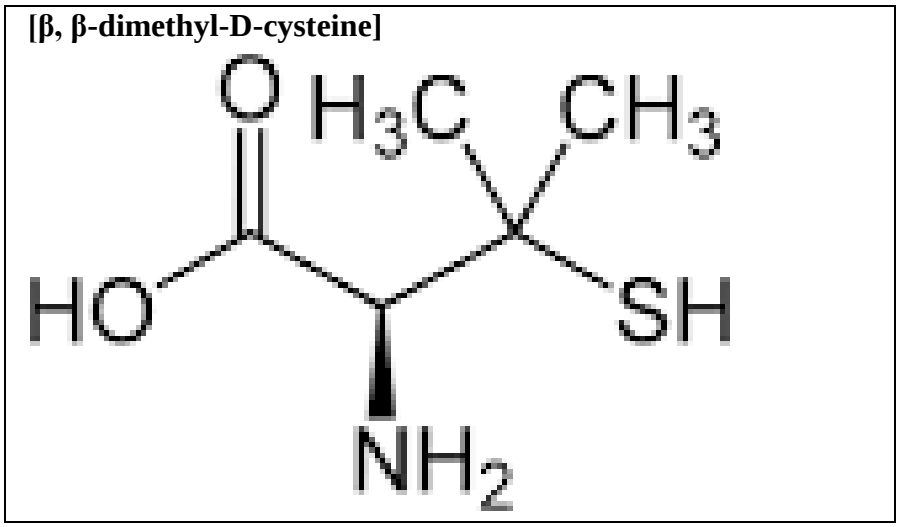
APPENDIX

Figures • Tables • Boxes

Figure 1. Structural formula of D-Penicillamine



Box 1. Dosages and use of D-Penicillamine in neonates.

BOX 1
Dosages and use of D-Penicillamine in neonates: *3 x 100 mg/kg bw./day intravenously 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.

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
ET = exchange transfusion

Table 2. Effects of D-Penicillamine vs. exchange transfusion (ET) in jaundice of term infants at 3-4 days of age.

	DPA-group	Controls
N (M:F)	**23 (11:12)**	**22 (12:10)**
Serum bilirubin (mg/dL)		
before interventions	**21.0**	**21.9**
8-12 hs after interv.	**20.0**	**17.9***
32-36 hs after interv.	**18.2**	**17.6**

***** significant difference

Table 3. 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)		
Before treatments	**9.8**	**10.0**
Peak bilirubin at 5-6 days	**12.2**	**12.8**
ETs	**5**	**6**

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

Box 2. Treatment of an infant with Rhesus-HDN without ET

Term infant boy was born as an 11. offspring of his other at 37. gestation with 3100 g bw. Cord blood: direct Coombs test strongly positive, bilirubin level: 4.2 mg/dL

Serum bilirubin	**Hemoglobin**
at 12 hs: 12.2	**119 g/L**
at 58 hs: 19.4	**108**
at 9 days: 2,8	**67 (50 ml PRBC)**

Th.: photherapy + DPA started at 12 hours of life for 5 days

Figure 3. Effect of a single dose of D-Penicillamine in 4-6 hours after intravenous administration.

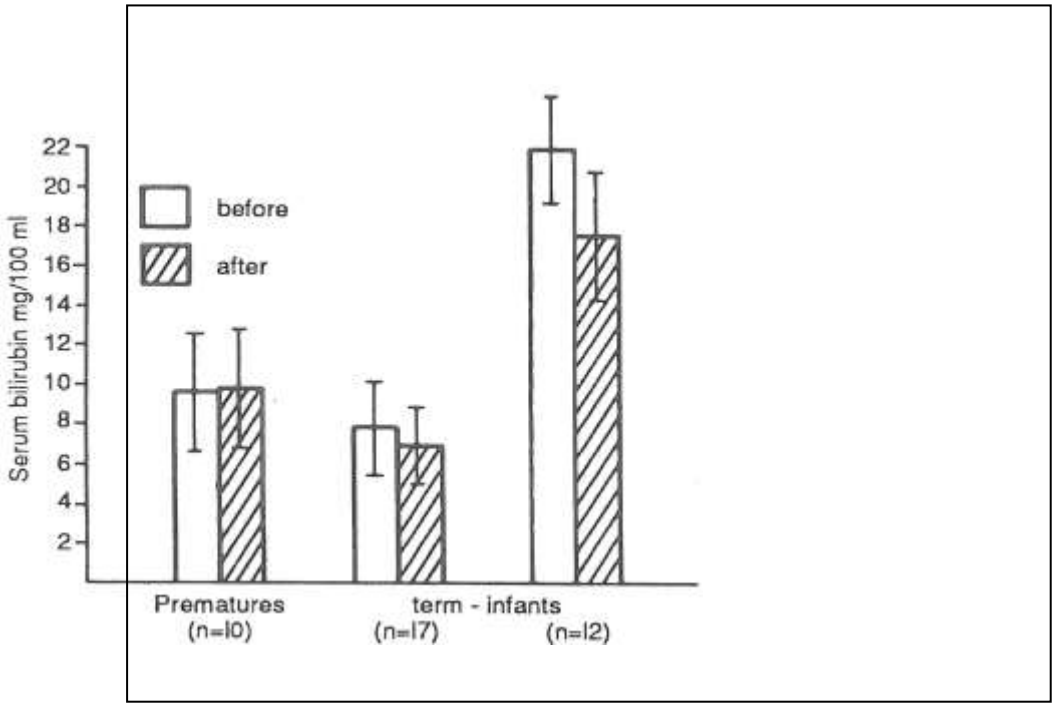


Table 4. History of D-penicillamine treatment of neonates < 1500 g birth weight

Dosage and administration	**First period (1973 – 1979) 300 mg/kg bw. IV for 3 days**	**Second period (1979 – 1980) 300 mg/kg bw. IV for 3 days**	**Third period (1981 – 1982) 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 5. 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**			**1**		

Table 6. 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**	**?**

Box 4. Oxidation of D-Penicillamin and the low molecular weight disulfid

BOX 4.
$2DP\text{-}SH + O2^{-} = DP\text{-}S\text{-}S\text{-}DP + H2O$

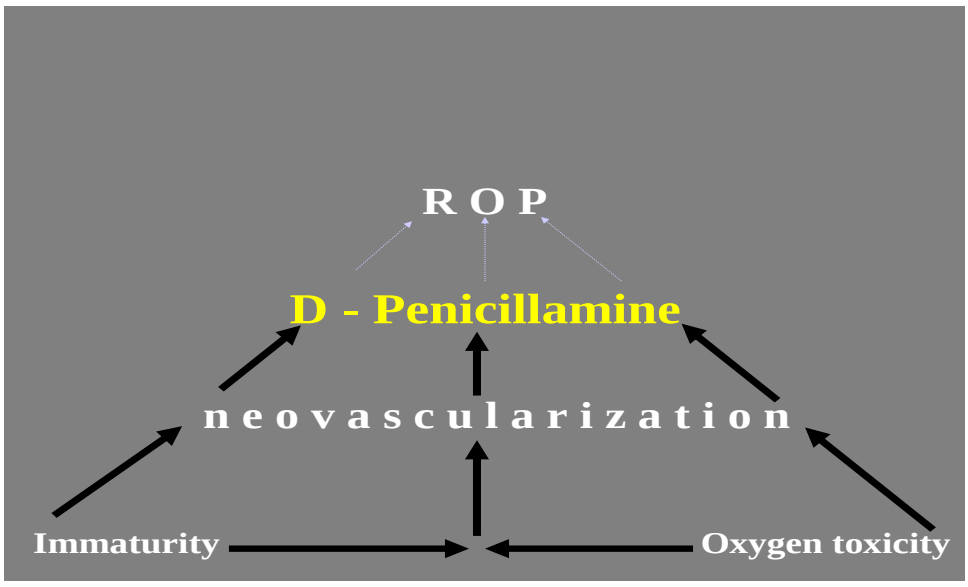
Box 5. Splitting of disulfide bridges of vascular endothelial growth factor by D-penicillamine

BOX 5
DP–SH + R–S–S–R ⇆ DP–S–S–R + R-SH **DP–SH + DP–S–S–R ⇆ DP–S–S–DP + R-SH**

Box 6. Copper (Cu) stimulates blood vessel formation

BOX 6
Cu is an obligatory cofactor of angiogenesis **Cu stimulates endothelial cell migration, and proliferation** **Cu activates vascular growth factors, and is required for collagen synthesis**

Figure 4. Proposal mechanisms of action of D- Penicillamine in the prevention of retinopathy of prematurity



Box 7. The incidences of side-effects of D-Penicillamine in rheumatoid arthritis (RA) and in the neonatal period

BOX 7		
	in RA (***long-term treatment***)	***in Neonates*** (***short-term treatment***)
• **dysgeusia**	**?**	
• **rash**	**very rare**	
• **proteinuria**	**no**	
• **leukopenia**	**no**	
• **thrombocytopenia**	**no**	
• **aplastic anemia**	**no**	
• **glomerulopathy**	**no**	
• **myasthenia gravis**	**no**	
• **pemphigus**	**no**	
• **Goodpasture syndrome**	**no**	

Box 8. Age-related difference in acute toxicity of D-Penicillamine in the neonatal period

BOX 8	**NEONATES (21 RATS)**	**ADULTS (20 RATS)**
LD_{50}	**> 4000 MG/KG (IP)**	**500 MG/KG (IV)**

Boksz 9. The two crucial questions of neonatal intensive care

BOX 9

- **„TO BE OR NOT TO BE…”?**
- **THE QUALITY OF LIFE?**

ABBREVATIONS:

AIDS - Acquired Immunodeficiency Syndrome
DPA - D-Penicillamine
ET - Exchange transfusion
FDA - Food and Drug Administration
HDN - Hemolytic Disease of the Newborn

HIV - Human Immunideficientia Virus
IV - Intravenously
NHBI - Neonatal Hyperbilirubinemia
PBRC - Packed Red Blood Cells
RA - Reumathoid Arthritis
RLF - Retrolental Fibroplasia
ROP - Retinopathy of Prematurity
SEBI - Serum Bilirubin Concentration
UDP- - Uridine Diphosphate (-glucuronyltransferase)
VEGF - Vascular Endothelial Growth Factor
VLBW - Very Low Birth Weight