



D-Penicillamine in the Neonatal Period: Possible Beneficial Effects on the Lethality of HIV 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 remarkably low incidence of retinopathy of prematurity (ROP) in the infants treated with DPA. Later, our studies were replicated in other institutes in Hungary, Poland, the 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-positivity due to vertical transmission with short-term DPA therapy (300 - 400 mg/kg/bw/day for 5-7 days). Therefore, we have a moral obligation to help the fight against HIV with this inexpensive (~ 30 USD/baby) drug in the neonatal period.

Keywords: D-penicillamine, neonatal hyperbilirubinemia, retinopathy of prematurity, HIV/AIDS therapy.

INTRODUCTION

When in the early 1970s, we reviewed the role of DPA in the treatment of NHBI¹, 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, serendipitously, to one of us (L. L.), while reflecting on the similarity of copper storage in Wilson's disease and neonates². It is well known that all neonates have increased concentration of copper in the 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 on only 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 in 1942³. It is now more than 50 years since Walshe⁴ 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 well⁹.

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 diseases)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 nowadays not available in the market and the per os preparation is produced by a few companies in the world.

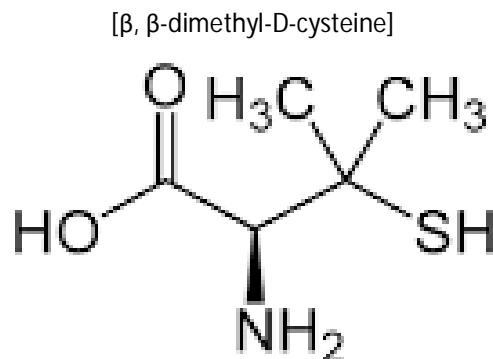


Figure 1: Structural formula of D-Penicillamine

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.

Clinical observations in the treatment of NHBI

Evidence from randomized trials is essential to inform rational choices made by health care providers¹¹⁻¹³. 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).

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

Table 4: 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 5: Infants enrolled in the study

Birth weight (g)	DPA-treated	Control
	All/Survived (%)	
751-1000	14/5 (35,7)	14/3 (21,4)
1001-1250	42/23 (54,8)	34/23 (67,6)
1251-1500	55/42 (76,4)	52/36 (69,2)
Total	111/70 (63,1)	100/62 (62,0)



Table 6: 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 7: 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	?

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 infants¹⁴. 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 group¹⁵. In another clinical study¹⁶ 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 signs 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 needed⁴⁸.

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**).

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 rats¹⁷. 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 hemolysis¹⁸.

The susceptibility of red cell lipids to autooxidation is about three times as high in the newborn as in adults¹⁹. *In vitro*, the preincubation with DPA resulted in a significant decrease of both the hemolysis and fluorescence of red cell lipid extracts^{20,21}.

In vivo, pretreatment with DPA has prevented the phenylhydrazine-induced lipid peroxidation in rats.

Malondialdehyde is a product of lipid peroxidation resulting in disintegration and disruption of biologic membranes. The binding of DPA to malondialdehyde may prevent this process²²⁻²⁵.



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 degradation²⁶. The 3 days of DPA treatment in the adult animals did not lead to any significant change in heme oxygenase activity²⁷. 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 Kappas²⁸. The high activity of heme oxygenase in the newborn could reflect the enzyme-inducing action of metals derived from the breakdown of fetal erythrocytes^{29,30}. Chelation therapy in neonates restores the normal activity of enzymes participating in heme metabolism.

Thus, chelating agents facilitate heme synthesis and inhibit heme degradation^{31,32}.

In the light of the foregoing we present our clinical observations in **Figure 2**. 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 babies¹⁶.

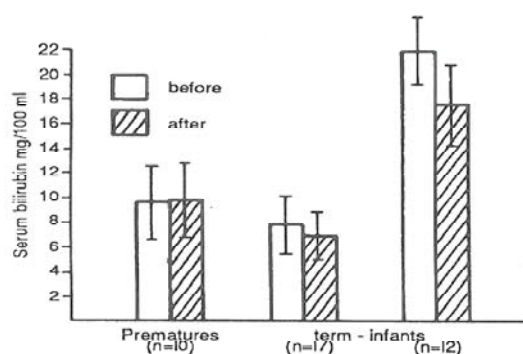


Figure 2: Effect of a single dose of D-Penicillamine in 4-6 hours after intravenous administration.

Very interesting and impressive **case reports** were

recently published by us³³.

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

Improved survival of low birth weight, premature babies in developing countries has increased the incidence of ROP³⁴. Regrettably, 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 gestation³⁵. According to a recent World Health Organization report³⁶, ROP is emerging as a major 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 procedures³⁷ 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 suppression are the alveoli^{38,39}.

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 considered as a paradigm of a multifactorial disease of the developing retinal circulation^{40,41}.

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)⁴². It was surprising that among DPA recipient 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 therapy⁴³. 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 occurrence 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

cicatrical form of the disease but also in the reduction of the acute stages⁴⁴.

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 (**Table 5**). 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 6**)⁴⁵⁻⁵⁰. 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 toxicity⁵¹⁻⁵³.

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 cicatrization⁵⁴.

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)^{55,56}.

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 pharmacology⁵⁷. In the **Table 7**, we demonstrate the results of our animal experiments regarding the age-related differences in effects of DPA⁵⁸. 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 level⁵⁹.

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 peroxidation⁶⁰⁻⁶². 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 encephalopathy⁶³.

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 1**)⁶⁴⁻⁶⁹.

Neovascularization

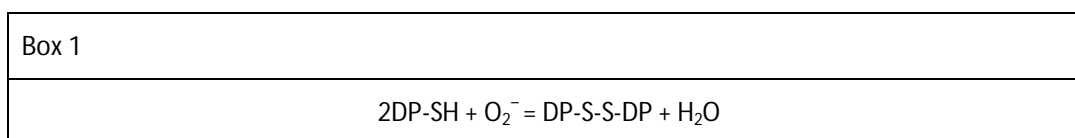
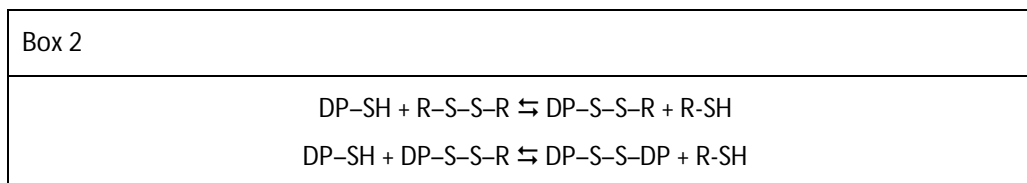
The pathophysiology of ROP understood to start with injury to the incomplete developing retinal capillaries. Once the developing vessels have been damaged, it is hypothesized that the retina responds with the production of VEGF stimulating neovascularization (which is the observable retinopathy) which may progress to neovascular membranes in the vitreous and subsequent scarring (cicatrix) and retinal detachment⁷⁰. 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 retinopathy⁷¹⁻⁷³.

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 2**)⁷⁴⁻⁷⁸.



Box 1: Oxidation of D-Penicillamin and the low molecular weight disulfide**Box 2:** Splitting of disulfide bridges of vascular endothelial growth factor by D-penicillamine**Box 3:** The incidences of side-effects of D-Penicillamine in rheumatoid arthritis (RA) and in the neonatal period

Box 3	
in RA (long-term treatment)	in Neonates (short-term treatment)
<ul style="list-style-type: none"> • dysgeusia • rash • proteinuria • leukopenia • thrombocytopenia • aplastic anemia • glomerulopathy • myasthenia gravis • pemphigus • Goodpasture syndrome 	<ul style="list-style-type: none"> ? very rare no no no no no no no no

Box 4: Age-related difference in acute toxicity of D-Penicillamine in the neonatal period

Box 4		
	NEONATES (21 RATS)	ADULTS (20 RATS)
LD ₅₀	> 4000 MG/KG (ip)	500 MG/KG (iv)

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 (Cu is an obligatory cofactor of angiogenesis, stimulates endothelial cell migration, and proliferation, activates

vascular growth factors, and is required for collagen synthesis)⁷⁹.

DPA as a heavy metal chelator

DPA was first use as a heavy metal chelator especially binding copper by its NH₂ group (**Figure 1**).

To sum up and over-simplify the mechanisms of action of DPA to prevent ROP can be seen in the (**Figure 3**).



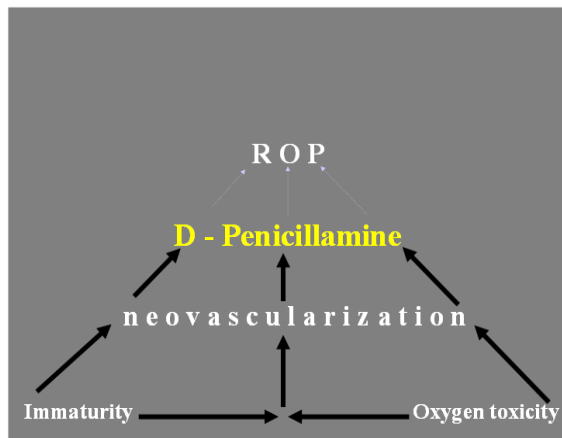


Figure 3: Proposal mechanisms of action of D-Penicillamine in the prevention of retinopathy of prematurity

Safety and tolerance of DPA, or else “nomen est omen”

The name of this drug has become frightening for doctors and, especially, for neonatologists, 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% adverse effects (BOX 3).^{80,81}

In rheumatoid arthritis there is a background of disturbed immune system in contrast in patients with Wilson’s disease and neonates with Wilson’s disease-like condition². 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 antigen⁸². Furthermore, we must stress that there are no immunosuppressive effects of this drug in the neonatal period, particularly in the course of a short-term therapy⁸³⁻⁸⁵. It is also noteworthy that there is a significant age-related difference in the acute toxicity of DPA administered parenterally to the animals (BOX 4).⁸⁶

The two crucial questions of neonatal intensive care: (1) “to be or not to be...”, (2) the quality of life⁸⁷. 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 advantage over controls in regards rehospitalization and ophthalmological outcome including ROP and other visual impairments⁸⁸⁻⁹⁰.

“Non-replication of the replicable”

Dr. William A. Silverman has written the above quoted title in his book-chapter⁹¹. He was the father of neonatal intensive care, and the author of a far-famed book⁹². Bill

proved to be our greatest supporter^{93,94} and he reviewed our research work in details, and insisted on the importance of replications.

We can say that until Silverman’s “declaration” only sporadic publications appeared in Hungarian and Polish journals⁹⁵⁻⁹⁹, 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 NHBI¹⁰⁰. As far as we know DPA therapy was recently tried in Mexico¹⁰¹ in the neonatal icterus, as well. Then, we published a provocative letter¹⁰² 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^{103,104} 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 trial¹⁰⁵ without any reduction in the number of ROP in the DPA-treated group. This controversial outcome was reflected in the Cochrane reviews concerning the prevention of ROP^{106,107}. 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 clear-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 burden¹⁰⁸, in the persistent pulmonary hypertension of newborn^{109,110} and probably in the treatment of HIV positivity due to vertical transmission^{111,112}. So, this is an ideal drug for the realization of a win/win approach¹¹³.

International replication, and the need for long-term 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^{114,115}. 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-5D¹¹⁶ 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 HRQOL¹¹⁷. 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 Németh¹¹⁸. 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 Analogue 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 well¹¹⁹. Prevalence of chronic health conditions did not deviate from predictable rate. The data of this pilot study is not sufficient for counting statistical significance. Further data analysis of 377 respondents are in progress¹²⁰.

CONCLUSION

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 expected¹²¹ – is very low in the infection of HIV- 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 DPA¹²².

The structural and functional properties of DPA make it suitable for exerting antiviral activity. This drug caused a marked inhibition of poly- virus-specific RNA and proteinsynthesis¹²³. 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*¹²⁴, (a single 100 mg/kg bw. IV administered DPA resulted in more multiple plasma concentration in premature infants)¹²⁵. So, this study has another promising idea wondering whether or not it is true that DPA has possible beneficial effects on the AIDS 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 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 approach in treating neonatal HIV or EBOLA infection. The newborn infants, especially the prematures, are suffering in an oxidative stress condition¹²⁶.
- It acts as a potent protease inhibitor in animal model¹²⁷.
- The copper metabolism in Wilson's disease and in newborn infants is strikingly similar: they both have large quantities of copper in the liver and low ceruloplasmine in the blood. It was previously found that cupric chloride, in the presence of a chelating agent, could inhibit the HIV-1 protease¹²⁸.
- 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 the intracellular transport of Zn and Cu ions¹²⁹.
- 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-replication could be inhibited by this drug¹³⁰⁻¹³².



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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Competing Interests statement

The authors declare no competing interests

Foot notes

Abbreviations:

AIDS - Acquired Immunodeficiency Syndrome

DPA - D-Penicillamine

ET - Exchange transfusion

FDA - Food and Drug Administration

HDN - Hemolytic Disease of the Newborn

HIV - Human Immunodeficientia Virus

IV - Intravenously

NHBI - Neonatal Hyperbilirubinemia

PBRC - Packed Red Blood Cells

RA - Rheumatoid 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

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