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D-penicillamine in the prevention of retrolental fibroplasia

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The effect of D-penicillamine on the occurrence of retrolental fibroplasia was analysed in 195 infants born in the years 1977—1980 with birth weights between 700 and 1500 g. Among 109 D-penicillamine treated infants a single one was found to have retrolental fibroplasia whereas of 86 without such treatment 10 developed severe cicatricial retrolental fibroplasia. It seems that in preventing hyperbilirubinaemia and oxygen toxicity the mechanism of action of D-penicillamine is a protection of biomembranes against lipid peroxidation.

Nearly 40 years have passed since Terry [33] had described retrolental fibroplasia (RLF) in premature infants. With the decreasing mortality and increasing survival rates of prematurely born infants, the risk of RLF is still a serious problem. Clinical and experimental evidence has suggested that oxygen toxicity is a major factor in the aetiology of RLF and bronchopulmonary dysplasia [11, 13, 31, 32]. Identification of agents or drugs to protect against oxygen toxicity may provide a means to reduce the incidence of these disorders.

D-penicillamine (DPA) for the control of neonatal hyperbilirubinaemia was introduced in our Department in March, 1973 [18, 19]. This treatment was especially effective in jaundice of haemolytic origin such as ABO or Rh incompatibility, and is used with phototherapy for the prevention of hyperbilirubinaemia of premature infants.

The present study was performed to draw attention to a new possible use of DPA, in the prevention of RLF.

Material and Methods

In a previous retrospective study, 407 premature infants who had received supplemental oxygen therapy during the period May, 1974, to December, 1978, were examined by indirect ophthalmoscopy. Eleven cases of RLF were diagnosed in that study. During the two and a half years prior to the development of our neonatal intensive care unit there was only 1 out of 158 in contrast to the 10 out of 249 cases during the following two years who were found to have cicatricial phase RLF. Every infant who developed RLF had a birth weight under 1500 g and a gestational age of 26 to 33 weeks. It was surprising that among babies treated with DPA to prevent hyperbilirubinaemia in the neonatal period of life there was only one case of RLF. The clinical data of the 193 infant survivors with a birth weight between 700 and 1500 g are shown in Table I. Two aspects have to be considered:

TABLE I

Distribution of very low birth weight infants receiving supplemental oxygen therapy and D-penicillamine treatment in the retrospective screening program

Birth wt g Gestational age, wks	DPA	Intensive care nursery		Total
		Before	After	
		n/RLF		
< 1500	—	46/0	86/10	132/10
26—33	+	25/1	36/0	61/1

n/RLF = Incidence of retrolental fibroplasia in the given groups

TABLE II

Survival rates of infants weighing less than 1500 g, in 1975—1980

	Year					
	1975	1976	1977*	1978*	1979*	1980*
Survival rate per cent	31.5	47.6	60.0	60.6	54.3	60.9

* Treated in intensive care nursery

first, it is obvious that DPA treatment was associated with a decrease in the incidence of RLF; second, only the cases in the marked quadrant can appropriately be compared i.e. the patients discharged after the development of intensive care nursery. The unequal division of cases probably indicates the hesitancy to withhold DPA treatment for premature infants in view of its presumed beneficial influence. So we decided that no additional infants should be placed in the "control" group, and all infants weighing less than 1500 g and requiring oxygen received DPA therapy during the period May 1979 — October 1980 [17a]. Our decision was supported by the following considerations. (i) For infants with respiratory difficulties at any time in the neonatal period, oxygen administration was always indicated. Unfortunately, at the same time we had no possibility to measure the arterial PaO_2 systematically. The method of oxygen treatment has remained unchanged since January 1977. Then, all prematures were assigned to routine (unrestricted) oxygen for the 18 months of the supplemental prospective study. As can be seen in Table II, during the past four years, the survival rate of infants with very low birth weight has improved significantly, largely because of their treatment in the intensive care nursery, and it tends to remain unchanged. (ii) Elevation of the unconjugated bilirubin level is a frequent occurrence in preterm babies. Although DPA itself has a moderate effect against hyperbilirubinaemia of prematures with a short gestational age (15), together with phototherapy it seems to control the plasma bilirubin level satisfactorily in very low birth weight infants too. (iii) Last, but not least, ethical aspects have also influenced our decision, RLF is namely a severe disease resulting in lifelong blindness.

DPA (Metalcaptase® — Knoll AG, Ludwigshafen, FRG) was applied intravenously in doses of 300 mg/kg body weight daily,

divided into 4 equal amounts, starting at 12—24 hours of life. The period of treatment varied between 2 and 5 days. There were 160 preterm infants with very low birth weight admitted to the neonatal intensive care unit from May 1, 1979, to October 31, 1980. All neonates required supplemental oxygen. Of these infants 91 (57%) survived. The infants were examined with regular neurologic and developmental evaluation in our neonatal clinic; 73 (81%) were then examined by indirect ophthalmoscopy with scleral depression. The rest of the patients (n = 18) could not be contacted and did not report at the clinic. Generally, the first fundus examination was scheduled at 3 to 5 weeks of age. If the initial finding was normal, the baby was examined monthly up to 6 months of age. The eye changes were graded according to Reese et al [28]. Gestational age was determined from the menstrual history and confirmed by the clinical criteria of Dubowitz et al [6]. The medical records of the 195 infants, 109 treated with DPA and 86 untreated, discharged between 1977—1980, were reviewed and the systematically tabulated clinical data were analysed by Student's t or the χ^2 test.

Results

The clinical data and neonatal morbidity factors of the 195 survivors with a birth weight between 700 and 1500 g are shown in Table III. These data indicate that the two groups of infants treated with or without DPA were well matched on the basis of birth weight, gestational age, and neonatal morbidity factors. Also, the neonatal factors associated with the inspired amount of oxygen showed no significant difference except for phototherapy in the DPA-treated and not in the control group (Table IV). The data for the 11 infants who developed RLF and survived the neonatal period are summarized in Table V. One case out of 109 DPA-treated infants was found to have RLF, whereas 10 out of 86 without such treatment developed severe cicatricial RLF. The difference was statistically

Table III

Clinical data and neonatal morbidity factors of all infant survivors with a birth weight of 700 to 1500 g, in 1977—1980

Clinical data	DPA-treated	Control	P value*
No. of babies	36 + 73 = 109	86	
Birth weight, g	1261±19.8	1242±20.0	N. S.
Gestational age, wk	32.6±2.8	32.4±2.3	N. S.
Apnoea once	10	8	N. S.
Apnoea several times	56	39	N. S.
Septicaemia	7	4	N. S.
Maximum weight loss, g	121±46	88±7	N. S.

* = Probability value derived from χ^2 test or Student's t test. N. S. denotes $P > 0.05$

TABLE IV

Factors associated with oxygen dose

Factors	DPA-treated (n = 109)	Control (n = 86)	P value
No. of infants treated with O_2. Duration of O_2 exposure (hours)			
$FiO_2 = 1.0$	58	39	N. S.
	2.7±0.5	3.2±0.8	N. S.
$FiO_2 = 0.6$	70	54	N. S.
	77.8±27.8	47.1±6.4	N. S.
$FiO_2 = 0.4$	109	86	N. S.
	61.1±5.9	50.2±5.3	N. S.
Exchange transfusions, (number)	26	25	N. S.
Phototherapy, (number)	56	22	<0.01
Respiratory aids:			
No. of infants treated with CPAP	60	49	N. S.
Duration of O_2 exposure, (hour)	49.1±6.1	51.1±6.5	N. S.
No. of infants treated with O_2 via mask	109	86	N. S.
Duration of O_2 exposure, (hour)	65.3±6.0	49.8±5.3	N. S.

CPAP = Continuous positive airway pressure
FiO_2 = Fraction of inspired O_2

significant by the X^2 test. In addition the DPA-treated RLF infants had more total hours of oxygen treatment than the control RLF babies.

DISCUSSION

The results suggest that DPA treatment in the neonatal period is associated with a marked decrease in the incidence of severe RLF among very low birth weight infants. Such a protective effect of DPA would be consistent with a radical-mediated mechanism of oxygen toxicity.

At present, hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), hydroxyl radical (HO^-), and singlet excited oxygen ($^1O_2$) are all considered to be possible agents of hyperoxic tissue damage. Oxygen radicals, in turn, participate in radical chain reactions causing lipid peroxidation, depolymerization of mucopolysaccharides, protein sulphhydryl oxidation and crosslinking, which lead to enzyme inactivation and damage of nucleic acids [5, 8, 17].

Protective agents act directly to eliminate the formation of toxic substances such as oxygen radicals. Examples of such agents are radical quenchers or antioxidants. A second type of protection occurs with compounds that induce tolerance by increasing the activity of enzymes

TABLE V

Infants with retrolental fibroplasia

Infant No.	Date of birth	Gestational age, wk	Birth wt, g	Cicatricial RLF OD/OS	Total hours O_2 FiO_2			Total hours O_2		Et.	DPA	Comment
					1.0	0.6	0.4	CPAP	Mask			
1	1977	28	1200	O/V	8	100	100	100	108	+	—	IRDS
2	1977	28	950	V/V	6	72	48	72	54	+	—	IRDS
3	1977	31	1500	V/V	—	48	48	48	48	+	—	IRDS
4	1977	29	1400	V/V	—	60	50	60	50	—	—	IRDS
5	1977	30	1200	V/V	—	—	50	—	50	+	—	Tachypnoea
6	1977	32	1420	V/V	6	84	20	84	26	+	—	IRDS
7	1978	26	1150	V/V	6	186	20	180	32	+	—	IRDS
8	1978	33	1300	V/V	—	40	10	40	10	—	—	IRDS
9	1978	32	1250	V/III	—	30	10	30	10	—	—	IRDS
10	1978	27	950	I/II	—	60	70	—	130	+	—	Sepsis
11	1979	29	1100	V/V	4	200	200	200	204	—	+	IRDS

Et = Exchange transfusion

that protect against oxygen. Several studies have provided clinical and experimental data demonstrating that DPA may act by both pathways of the antioxidant defense mechanism.

I. DPA as an oxygen radical scavenger

(i) DPA, chemically dimethylcysteine, like other sulphhydryl compounds, can protect tissues from oxygen toxicity and it is related to its free radical scavenging SH group [7, 21, 27].

(ii) Enzymes which catalyse the dismutation of O_2^- are metallo-proteins termed superoxide dismutases. Lengfelder and Elstner [22] found a superoxide dismutating activity of the red-violet-copper-DPA complex. Recently it has been reported by Robertson and Fridovich [29] that the complex does not catalyse the dismutation of O_2^-. It is, however, not entirely stable. Its gradual decomposition releases Cu(II) or smaller complexes of DPA, and these catalyse the dismutation of O_2^-. In any case, if such copper complexes are formed during DPA treatment, this may offer protection against free radicals. Our previous animal experiments showed that prolonged hyperoxia caused an increase in superoxide dismutase activity while DPA was found to inhibit the inductive effect of oxygen. These data suggest that DPA may provide sufficient antioxidant protection to prevent the usual biochemical response to hyperoxia [14].

(iii) An undefined part of the drug effect might result from its chelating properties since trace metals, especially copper and iron, are potent catalysts of lipid peroxidation [1, 3, 16, 34]. This may be the most probable hypothesis, for it is well-known that the accumulation of trace elements is higher in the newborn's organs than in those of adults [30].

(iv) DPA, as other thiols, has a great tendency to form mixed disulphides with disulphide-containing serum proteins, and through these oxidations it may have a protective, reducing effect on free radicals and act as a hydrogen donor [25].

(v) DPA satisfies several criteria of radioprotection. It is a dose-modifying agent whose dose reduction factor is directly proportional to the concentration of the drug, and it affords protection when administered before, but not after, irradiation [35, 36]. Based on the report of Gerschman et al [9], it has been widely accepted that irradiation and oxygen exposure produce at least some of their lethal effects through one common mechanism, the formation of oxidizing free radicals. In addition, in our experiments the radioprotective effect of DPA was significantly greater in 3—4 days old mice than in adult animals [20].

(vi) In several previous reports we have shown that DPA given intravenously to newborns reduces the plasma bilirubin concentration or prevents the increase usually seen during the first few days of life. There are a number of observations to show that bilirubin is a photodynamic agent, i.e. in the presence of oxygen

and light it produces $^1O_2$ and peroxidation of unsaturated membrane lipids [4, 10, 12].

II. DPA as an inductor of enzymes contributing to antioxidant defense

(i) Administration of DPA may liberate some of the protein-bound glutathione and thus increase the concentration of free glutathione. This thio tripeptide plays an important role in the stability of cell membranes and in protection against damage from superoxides and free radicals [26].

(ii) In a recent study [24] we have examined how in vivo treatment affects the enzymes of peroxide metabolism in the liver of newborn rats and in the tissues of adult rats. It was found that the drug acted in different ways in newborn and adult rats. In both cases DPA caused an increase in the liver microsomal cytochrome P-450 activity and a decrease in lipid peroxidation. As regards the enzyme changes, DPA treatment led to an increase in liver peroxidase and catalase activity only in newborn rats. These findings are in accordance with our previous observation that DPA shortens the hexobarbital sleeping time in newborn but not in adult rats.

The age related differences in the drug effect can well be explained by the data of Maines and Kappas [23]. According to their studies, metal ion induction of haem oxygenase, a rate-limiting enzyme of haem degradation, is accompanied by various disturbances of haem metabolism including alterations in delta-amino-laevulinate synthetase activity, decreased microsomal contents of haem and other haem proteins. When metals are complexed with cysteine or glutathione, their ability to alter the metabolism of haem is totally blocked. The high activity of haem oxygenase in the newborn could reflect the enzyme-inducing action of metals derived from the breakdown of fetal erythrocytes. Chelation therapy in neonates restores the normal activity of enzymes participating in haem metabolism. Briefly, chelating agents facilitate haem synthesis and inhibit haem degradation, thus boosting the newborn's liver to the adult detoxifying level. Since the enzymes which play an important role in antioxidant defense and drug metabolism (peroxidases, catalase, cytochrome P-450) are haem proteins, it is likely that the prevention of hyperbilirubinaemia and of oxygen toxicity by DPA rests on a common mechanism, the protection of biomembranes against lipid peroxidation.

Finally, we wish to emphasize that although DPA was administered to the babies in uncommonly high doses as compared to those applied in adults, no serious side effects have so far been recorded in more than 2000 infants treated in the last eight years. Furthermore, on the basis of a follow-up study carried out at 3—4 years of age we could demonstrate that DPA had no harmful long-term side effects. Neither had the drug a bilirubin displacing effect on the albumin-bilirubin complex [2]. Conse-

quently, concerning risk *vs* benefit of DPA therapy of neonates, the data of the present study provide support for conducting further controlled clinical trials.

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