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Neonatal oxygen toxicity and its prevention: D-penicillamine offers benefits without harmful side-effects

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Oxygen toxicity in the newborn and the possibilities of its prevention are discussed. The role of active radicals developing from molecular oxygen, lipid peroxidation and its prevention by antioxidants, oxygen toxicity and its importance in neonatology, and facilities for overcoming hyperoxia of the newborn are considered. The antioxidant effects based on experimental and clinical observations of vitamin E and D-penicillamine are described. Since the introduction of D-penicillamine therapy the incidence of retrolental fibroplasia has steeply decreased. It is emphasized that in the neonatal period the drug has no adverse effects.

Oxygen (O_2) is indispensable for the highly organized functions of life. Oxygen therapy may be life-saving in hypoxia, but hyperoxia may lead to severe damage to cells and tissues.

The role of active radicals arising from molecular oxygen

During inspiration, O_2 dissolved in the lipids and the water of lung tissue diffuses through the alveolar membranes into the capillaries where it is bound to the haemoglobin of the erythrocytes; oxygen is then carried by the blood to the various tissues. It is well known that O_2 is indispensable for a large number of biochemical processes working at cellular and tissue levels. Most oxygen undergoes the four-electron reduction carried out by mitochondrial cytochrome oxidase; the end product of this process is water [12]:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Reduction by two electrons performed by other enzymes results in hydrogen peroxide

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$$

while a reaction using one electron leads to the superoxide radical

$$O_2 + e^- \rightarrow O_2^-.$$

In addition, O_2^- is produced by auto-oxidation of normal cellular components like thiol derivatives, haemoglobin, or epinephrine [67, 69, 70]. The bactericidal effect of O_2^- developing during phagocytosis by polymorphonuclear leucocytes is a characteristic property [3, 85]. H_2O_2 is comparatively well tolerated by most aerobic organisms in spite of its much higher reactivity to biological molec-

ules. The compound may be harmful if it is transformed to hydroxyl radical (·OH) in the presence of certain metal ions [33]. This radical is extremely aggressive. It may also be formed during the reaction of O_2^- and H_2O_2 catalyzed by iron chelates [24, 34]:

$$O_2^- + H_2O_2 \xrightarrow{Fe} 2\cdot OH + O_2$$

The interaction between various oxygen and peroxide radicals may result in a very reactive oxygen derivative toxic to most biological systems, in singlet oxygen ($^1O_2$). In this radical, one electron of the oxygen is pushed to a high energy orbit [48, 91].

H_2O_2, O_2^-, $^{\cdot}OH$ and $^1O_2$ are the main factors responsible for the tissue damage provoked by hyperoxia; "oxygen radical" is an oversimplified common term for them.

Lipid peroxidation and its biological consequences

According to the most accepted hypothesis, O_2 toxicity is provoked by free radicals arising from O_2 and by other active oxygen metabolites; their effect is mediated by increased lipid peroxidation (LP) of biological membranes, thus leading to specific cell damage [25, 67]. Oxidability of fatty acids depends on the number of double bonds, i.e. only unsaturated fatty acids (linolic acid, linolenic acid, arachidonic acid, etc.) are prone to peroxidation. Cellular membranes, containing a considerable quantity of unsaturated fatty acids, are especially vulnerable to peroxidative damage [16, 81, 99]. ·OH and probably also O_2^-, react with the lipid components of biological membranes; this results in peroxide (ROO.) and hydroperoxide (ROOH) radicals of lipids and in other compounds like malonyldialdehyde [5, 17, 47]. Malonyldialdehyde forms covalent bonds with the free amino-groups of proteins, lipids and nucleic acids, and on this reaction is based the chemical detection of LP precesses [9]. This chain reaction, initiated by the free oxygen radicals is catalysed by heavy metals, especially iron, and copper at several levels [51, 53, 99] leading to a loss of the integrity of membranes of erythrocytes, mitochondria, microsomes and lysosomes, to inactivation of enzymes in these structures and to damage to nucleic acids [50, 65, 71].

LP may accompany a number of physiological and pathophysiological events. Such normal physiological processes are prostaglandin synthesis, phagocytosis and aging [3, 37, 40, 65, 85]. Certain experimental and clinical observations have indicated the damaging effect of LP in haemolytic anaemia, dysfunctions of reproduction, certain pulmonary disorders, muscular atrophies, liver necrosis, encephalomalacia, atherosclerosis, testicular atrophy, diabetes mellitus, irradiation disease, postresuscitation syndrome, mutagenesis, carcinogenesis [16, 18] and other conditions including certain neonatal disorders to be described in this paper.

Antioxidant prevention

The antioxidant defence mechanisms are indispensable in maintaining life since the potentially destructive free radicals are continuously produced in the organism and they keep combining with each other [28]. The first line of defence consists of various physiological barriers hindering the oxygen to react with the tissues while entering from the alveolar space into the pulmonary capillaries and carried by haemoglobin to the cells [36]. The second type of defence is bound to specific enzymes: superoxide dismutase, catalase, peroxidases, glutathione and its enzyme system, and the NADPH producing system closely coupled to the hexose-monophosphate shunt [87, 106]. Since these enzymes exhibit activity induction during adaptation to hyperoxia, their function will be described in some detail.

Superoxide dismutase (SOD)

This metalloprotein enzyme has been found in three main forms. In eukaryotic cells the metal is copper and zinc in the cytoplasm, and manganese in the mitochondria. A third type of SOD contains iron and occurs in a number of bacteria. Their main task is to enhance the dismutative inactivation of O_2^- radicals produced during auto-oxidation of various biological components or arising during enzymatic processes. The dismutation can be described by the reaction,

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

As a result of this reaction, O_2^- is rapidly removed before it could induce direct cellular damage or lead to production of aggressive $^{\cdot}OH$ radicals by combination with H_2O_2 [25, 66, 100].

Catalase and other peroxidases

These are enzymes of haem-protein nature. Their main biological role consists in the elimination of H_2O_2:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Although H_2O_2 itself participates in LP at a low degree and slow reaction rate, its elimination is imperative because it reacts with O_2^- in the presence of metal ions, leading thus to $^{\cdot}OH$, the most dangerous radical from the aspect of oxygen toxicity [27, 33].

Glutathione and NADPH producing systems

Glutathione is a thio-tripeptide. A high concentration of its reduced form (GSH) and a low concentration of its oxidized form (GSSG) are essential in the maintenance of animal life. The antioxidant effect of GSH is exerted by two routes: it prevents the oxidation of sulfhydryl groups of the proteins by serving as a substrate for the oxidants; in addition, GSH is a substrate of the enzyme glutathione peroxidase which inactivates both H_2O_2 and lipid peroxides, stopping thus the chain reaction initiated by the oxygen radicals [35, 68]. The optimum GSH–GSSG ratio is maintained by the enzymes glutathione reductase and glucose-6-phosphate

dehydrogenase. The latter forms an essential step of the hexose-monophosphate shunt. Its production is greatly enhanced by the NADPH (reduced nicotine amide adenine dinucleotide phosphate) system, supporting in this way the important biosynthetic reactions necessary for the reparation of tissues damaged by lipid peroxidation [11].

The third line of antioxidant defence comprises endogenous antioxidants, the adequate tissue level of which offers satisfactory protection against oxygen toxicity. These compounds are vitamin E, selenium, sulfur containing amino acids, vitamin C [27, 97], and transferrin and coeruloplasmin, proteins performing the task of extracellular transport of iron or copper [19].

The special situation of neonates and low-birth-weight newborns

As shown above, oxygen toxicity is the resultant of two biological processes acting in two opposite directions. On the one hand, production of free radicals and the chain reaction initiated by them would tend to lead to disintegration of the cell and the cellular organelles. On the other hand, there exists a sophisticated defence system acting at several levels that prevents or even repairs the tissue damage. Under physiological conditions there is an equilibrium between the two systems; the identical rate of production and elimination secures only the "normal" function of lipid peroxidation. Pathological newborns and preterm infants are, however, in a peculiar situation. In these infants and to some extent in normal term babies as well, a disequilibrium resulting in increased vulnerability to oxygen toxicity may develop during adaptation to extrauterine life. In addition, defence mechanisms against the oxygen radicals are also immature in them. There are two main reasons for this increased vulnerability, (i) an increased exposure to situations of prolonged oxidative stress; (ii) deficient defence at all levels.

Ad (i)

1. Before and during birth the newborn may be exposed to maternal and fetal injuries resulting in an increased rate of LP: EHP-gestosis, intrauterine infections, various maternal diseases, placenta praevia, premature rupture of the membranes, exposure of the mother to drugs and radiation, especially tocolytics, procedures during labour like oxygen inhalation, anaesthesia, analgesia, oxytocin etc. [89].

2. The greatest risk is oxygen therapy some time after birth, frequently applied as a life-saving measure necessary for the prevention of sequelae of hypoxia [42]. The most vulnerable individuals in this respect are babies of very low birth weight and newborns in whom the partial tension of arterial oxygen continuously exceeds the tolerable level. For this reason, in newborn units all over the world attempts at measuring arterial oxygen tension are made to avoid prolonged hyperoxaemic

periods [49, 96]. Unfortunately, only partial success has been achieved as even in intensive care units not all the grave sequelae of oxygen toxicity can be avoided [83].

3. A frequent damaging factor is hyperbilirubinaemia. Apart of its unfavourable effect on the central nervous system [63], bilirubin has been shown to be a photodynamic agent i.e. it produces $^1O_2$ in the presence of oxygen and light, thus enhancing the peroxidation of unsaturated fatty acids [15, 29, 32].

4. Certain therapeutic measures destined to surmount neonatal hyperbilirubinaemia like phototherapy and exchange transfusion may also be potentially harmful in this respect. Phototherapy enhances the isomerisation of bilirubin into rapidly eliminated and less toxic compounds. This process is accompanied by an increased production of $^1O_2$. This radical has a minor role in bilirubin oxidation but it may be responsible for the potential hazards of phototherapy: damage to DNA, mutagenesis, carcinogenesis, sexual dysfunction, etc. [14, 93]. Similarly, exchange transfusions and transfusion of adult blood may increase the oxygen toxicity by the lower oxygen affinity of adult haemoglobin compared to that of fetal haemoglobin [13].

5. Septic and hypotensive conditions, including the idiopathic respiratory distress syndrome (IRDS), resuscitation and reparation after resuscitation increase the risk of oxygen damage. In chronic and acute inflammations the polymorphonuclear leucocytes, macrophages and monocytes produce a considerable amount of O_2^-; this radical has a direct bactericidal effect and promotes the formation of the superoxide dependent chemotactic factor and the production of $^{\cdot}OH$ radicals. Both compounds have a favourable effect in the killing process directed against the bacteria and other invasive agents [3, 85]. In the immature organism, however, with its deficient antioxidant defence, the aggressive radicals may damage the cellular integrity.

6. Certain drugs may increase the proneness to oxygen toxicity. Some have a direct sensitizing effect in favour of oxygen toxicity, e.g. riboflavin, methylene blue, salicylates, etc. [92]. Others enhance the albumin-bilirubin dissociation or weaken the activity of glucuronyl transferase thus promoting bilirubin and oxygen toxicity at the same time [63]; sulfonamides and chloramphenicol are in this group. Finally, artificial ventilation and X-ray exposure, not infrequent in modern intensive care, directly lead to overproduction of harmful oxygen radicals [4].

Ad (ii)

1. In newborn babies the physiological barriers lining the internal route of oxygen transport are immature. Immaturity and gestational age show a direct correlation. Size, weight, alveolarity, collagen content, concentration of the pulmonary surfactant, the proportion of interstitial elements, capillary microcirculation, the number of alveolar epithelial

cells, respiratory capacity and compliance of the lung all differ from the situation prevailing in the baby born at term [41].

2. The activity of specific antioxidant enzymes gradually increases during gestation and in the early postnatal period. The most potent inductor of this activity is the oxygen itself, and hyperoxia speeds up the enzyme adaptation process [1, 6, 107]. This may explain the finding that under prolonged oxygen exposure newborn animals survive much longer than adult animals; this does not imply that they do not suffer permanent tissue damage. Similarly, pretreatment with antioxidants abolishes the usual biochemical reaction to hyperoxia [103]. Our own experiments with SOD on the effects of D-penicillamine resulted in similar findings [46].

The neonatal insufficiency of the enzymatic defence is markedly promoted by heavy metals, especially by iron and copper ions. These latter accumulate in a considerable degree in the fetal tissues during the last weeks of gestation [88]. After birth an additional unfavourable factor is the increased haemolysis of fetal erythrocytes. The metal ions directly influence the oxidative processes and, in addition, they inhibit the activity of delta-aminolaevulinic acid synthetase, an enzyme governing the production of haeme-containing proteins like catalase, peroxidase and cytochrome P-450. They also stimulate haemoxygenase, an enzyme initiating haeme catabolism [62].

3. Newborn babies, especially very low birth weight preterm babies, are insufficiently supplied by endogenous antioxidants like vitamin E, selenium, transferrin and coeruloplasmin [23, 38].

Neonatal disorders related to oxygen toxicity

These disorders are specific to the newborn period. They have a multicausal aetiology, their common feature being the chain reaction leading to tissue damage, initiated by free oxygen radicals.

Haemolytic diseases

Auto-oxidation of the newborn's erythrocytes proceeds at a rate three to five times higher than in adults [94]. An especially unfavourable situation is encountered in babies affected by congenital deficiency of glucose-6-phosphate dehydrogenase, pyruvate kinase or glutathione reductase [95].

Retrolental fibroplasia (RLF)

Terry [98] gave the first description of RLF of premature babies in 1942. The pathology of the condition was clarified 10 years later [26] when it was also recognized that the principal cause was an uncontrolled high oxygen supply [10, 80]. Although this view has repeatedly been confirmed, no efficacious prophylactic or therapeutic measure has been available and RLF has become the heaviest burden of modern intensive care treatment [90]. The increasing

survival rate of very low-birth weight infants has led to an increase in the number of blind people [42, 89, 96].

Bronchopulmonary dysplasia (BPD)

A better understanding of the pathomechanism of IRDS has led to the introduction of continuous positive airway pressure (CPAP) ventilation, bringing about a dramatic improvement in the prognosis of the disease [31].

However, BPD, a new clinical entity has appeared on the scene [75]. The condition is a progressive pulmonary fibrosis leading to severe respiratory failure; it is caused, in addition to oxygen therapy, by prolonged mechanical ventilation and patent ductus arteriosus [104].

Hyaline membrane disease or IRDS

This condition is specific to preterm babies and pathological newborns and leads to alveolar hyaline membrane production. The process is related to oxygen toxicity in several respects. Increased catabolism due to the severe condition of the newborn may increase the production of free radicals, and the therapeutic efforts may directly enhance LP. Recently it has been shown that the peroxidative chain reaction triggered by oxygen radicals may play a primary role, since the hyaline membrane is basically a LP product [8]; it can be induced experimentally by ionizing radiation and type II pneumocytes producing the surfactant are damaged by oxygen radicals [2].

Possible methods for surmounting the adverse effects of hyperoxia

Theoretically, there are two possibilities to increase the defence against oxygen toxicity. Antioxidant agents are substances directly neutralizing the free radicals. Other compounds enhance the activity of enzymes counteracting the effect of oxygen radicals and repair the damage caused by lipid peroxidation. Vitamin E is a good example of antioxidants while D-penicillamine plays an active role in both respects.

Vitamin E

Vitamin E has been known for more than fourty years [22] but the exact mechanism of its antioxidant effect has not been clarified [82]. Vitamin E deficiency was observed to lead in certain animals to disorders probably due to increased auto-oxidation, i.e. infertility, encephalomalacia, muscle dystrophy or demyelinisation. Human hypovitaminosis E occurs only in preterm infants or in chronic fat malabsorption [30, 105]. Several authors attribute a primary aetiological role in neonatal oxygen toxicity to the low vitamin E concentration in the preterm babies' tissues [78, 79]. Vitamin E has been applied for its antioxidant effect by several authors; some reported on success in the prevention of BPD [20] or of RLF [45, 39], others have not been able to observe any convincing effect [42, 44, 84, 89, 102]. We feel that Dormandy's remark still holds [18]: "Forty years after its discovery,

vitamin E remains a biochemical Don Basilio, not to be trusted or believed but impossible to dismiss".

D-penicillamine (DPA)

In previous studies we have shown that intravenous DPA is a potent drug in the treatment and prevention of neonatal hyperbilirubinaemia [56, 58]; it was especially effective in haemolytic disease due to incompatibility in the Rh and ABO systems [57]. Its use combined with phototherapy allowed to avoid exchange transfusions in preterm babies affected by hyperbilirubinaemia [7]. A new impetus to our research was the observation of a reduced incidence of RLF among very low-birth-weight infants treated with DPA during the newborn period. This retrospective observation was later confirmed by a comparative study of preterm babies weighing less than 1500 g at birth, 109 of whom were treated with DPA and 86 without the drug [54, 55]. The two groups were shown to be comparable in a number of perinatal factors including those known to increase oxygen toxicity. The incidence of cicatricial RLF was 0.9 % in the treated group while it was as high as 11.6 % among the babies not treated with DPA. Since our first observation in 1979, all preterm babies weighing less than 1500 g at birth and undergoing oxygen therapy have been given DPA treatment. Table I shows our recent results. It is seen that while intensive perinatal care has brought about a marked increase in the survival rate of very low-birth-weight infants, this was accompanied by a sharp increase in the incidence of loss of sight due to RLF. On the other hand, ever since the introduction of DPA treatment, only one case of RLF has been observed and none during the last two years. Mortality, a sensitive indicator of adequate oxygen therapy, has been unchanged during the same period.

I. DPA directly neutralizes free oxygen radicals. DPA is dimethylcysteine and like all compounds contain-

TABLE I

Number, mortality rate and incidence of retrolental fibroplasia in premature infants weighing less than 1500 g at birth, 1977–1981

Year	Intensive neonatal care				
	Without DPA		With DPA		
	1977	1978	1979	1980	1981
All cases	90	142	140	138	134
Deaths, No.	36	56	64	54	51
per cent	40.0	39.4	45.7	39.1	38.1
No. of survivors	54	86	76	84	83
RLF, No.	6	4	1	0	0

DPA was administered to all preterm babies from May, 1979.

ing SH-groups it is a hydrogen and electron donor [21, 60, 74]. It has been shown that the red-violet complex of copper and DPA exerts SOD activity [61]; other authors, however, assumed that not the complex itself but other copper complexes of low molecular weight liberated by gradual decomposition of the whole complex are responsible for the dismutation of O_2^- [86].

It is highly probable that the antioxidant property of DPA is largely due to its chelating capacity, binding heavy metal ions known to catalyse LP [43].

As every thiol-derivative, DPA forms mixed disulfide bonds with serum proteins containing sulfhydryl groups; during this process it undergoes oxidation and in this way prevents the development of free radicals [73].

It has been shown [59] that the radioprotective property of DPA is more expressed in suckling mice than in adult animals.

The fact that the drug markedly decreases the serum bilirubin level, seems to point to its potent antioxidant activity [13].

DPA treatment leads to an increased intracellular concentration of free glutathione [72].

II. DPA increases the activity of enzymes participating in the antioxidant defence of newborn babies. This may explain the age dependent differences in the action of DPA. It was shown earlier [64] that in newborn animals DPA enhances the activity of hepatic peroxidase and catalase while such an effect could not be observed in adult animals. Similar results were obtained concerning lipid peroxidation in erythrocyte membranes, induced by hydrogen peroxide or phenylhydrazine [76].

The age dependence of the DPA effect was shown in rat experiments by measuring hexobarbital sleeping time. A marked reduction of the parameter by DPA treatment could be achieved in newborn animals. In addition, DPA induced an increase in microsomal cytochrome P-450 and led to multiplication of mitochondria in the liver cells only in newborns. In newborn animals, DPA inhibits haemoxygenase, the enzyme initiating haeme catabolism and so it not only inhibited bilirubin production but also ensured the persistence of high levels of the antioxidant enzymes of haemeprotein structure [77].

Recently, direct evidence has been produced of the sight protective effect of DPA, demonstrating that the drug significantly inhibited the proliferative processes in the corpus vitreum [101].

DPA treatment in the newborn may deeply affect some complex biochemical events and as a result it may shorten the postnatal adaptation period and reduce in that way the incidence and severity of some pathological phenomena appearing characteristically in the course of adaptation. In spite of this, many paediatricians are reluctant to apply DPA in newborn babies. This may be attributed to a fear of the unpleasant and dangerous side-effects of pro-

longed DPA therapy observed in some patients affected by rheumatoid arthritis [52]. It must, however, be emphasized that preterm infants are not small adults with rheumatoid arthritis. Even a high dose of DPA administered for some days has no acute side-effect in babies nor could any chronic side-effect be detected by prolonged observations. The drug had no effect on the albumin-bilirubin complex [7], and none of the outward effects described in adults have ever been encountered in our material. To date a total of 2500 newborn babies has been treated with DPA during the last 9 years, without any complication whatever.

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