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Damaging effect of free radicals liberated during the reduction of oxygen: its influencing by drugs

G. Balla, Anikó Makay, Zsuzsa Pollár, B. Matkovics, L. Lakatos and L. Karmazsin

Department of Paediatrics, University Medical School, Debrecen; Biological Isotope Laboratory, József Attila University, Szeged; National Centre of Medical Information, Budapest

An experimental model has been elaborated for measuring the effect of various factors on oxygen toxicity in newborns. The preventive effect of the compounds with antioxidant properties, D-penicillamine, 6,6'-methylene-bis (2,2,4-trimethyl-1,2-dihydroxyquinoline), and vitamin E, on damage caused by hyperoxygenation was judged by measuring superoxide dismutase activity and the tissue content of malonyldialdehyde. Both compounds were found to possess antioxidative effects by either method; their use for therapeutic purposes is thus recommended.

Oxygen is the third most common element in nature. Five per cent of the human organism as a total and 67.8% of its aqueous compartment consist of oxygen. Molecular oxygen gas, playing a central role in vital functions, is a homogeneous, ubiquitous compound with excellent diffusibility. Most physiological reactions of molecular oxygen are mediated by enzymes. Reduction of molecular oxygen in aqueous solution under the effect of various catalysts leads to liberation of certain free radicals like superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl (OH.); their biological role has become a central topic of medical research during the last decade.

Some of the radicals arising from molecular oxygen play an important role in cellular respiration, metabolism and multiplication. Electron paramagnetic resonance spectroscopic studies revealed that these bioradicals are indispensable in subcellular reactions of mitochondrial oxidation. The free radical concentration shows a gradual increase during mammal organogenesis; after completion of this early embryogenic phase their concentration decreases. Figure 1 illustrates the production of free radicals as a result of electron transport, drugs, enzymes, air pollutants and ionizing radiation; in addition, the effects of enzymes on the free radicals are also shown. Free radicals induce autooxidation which is a typical chain reaction of free radicals. These are highly reactive atomic groups without electric charge, possessing an electron of paramagnetic property without a mate. They can attach to

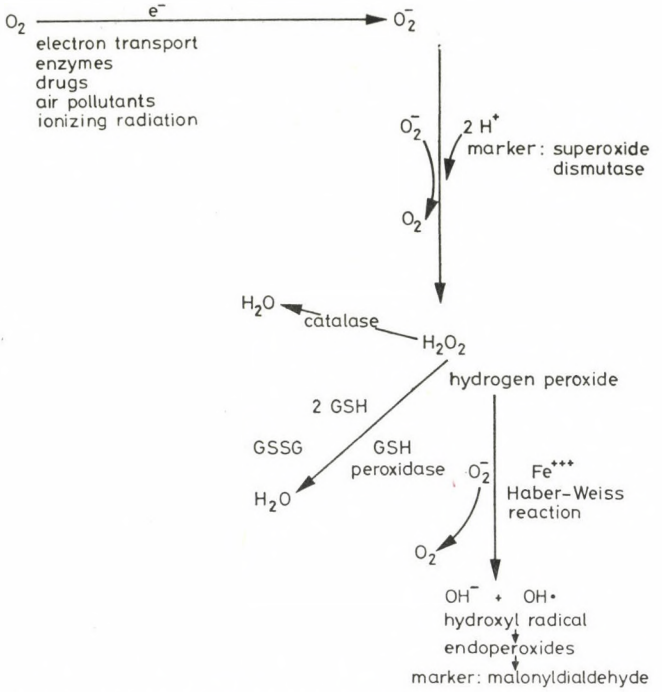

Fig. 1 Development of free radicals as a result of electron transport. The effect of certain enzymes on these radicals

carbon, nitrogen, halogen or other atoms.

Auto-oxidation proceeds through several phases.

a) Slow induction period: a radical arises by detachment of a hydrogen from a methyl group activated by an adjacent unsaturated bond.

b) The radical reacts with oxygen resulting in a peroxide radical.

c) The peroxide radical captures a hydrogen atom from the activated methyl group of another molecule, and hydroperoxide develops.

d) This latter radical, reacting with oxygen, is transformed to peroxide; this in turn reacts in the same way as described above, thus leading to a chain reaction.

Auto-oxidation in the living organism is a frequent event on membranes rich in lipids. Reactions resulting in liberation of free radicals may be enzymic or non-enzymic. No notable auto-oxidation occurs in the healthy human organism; in fact, the lipids resist oxidation for several hours after death. This points to the existence of extremely effective bio-auto-oxidants within the human organism [1, 6]. One of the most important among them, vitamin E, has been found in decreased quantity in prematures; in several diseases characteristic of premature age, a deficiency of bioantioxidants has indirectly been shown by administration of synthetic antioxidant compounds [12, 14].

Among the biological reactions mediated by oxygen radicals, liberation of superoxide anion during cellular aerobic glycolysis plays an important role in the bactericidal effect during phagocytosis [2, 7, 13, 20, 21].

In paediatrics, a metabolic disorder induced by the superoxide anion radical has gained special importance: elevated oxygen concentration in bradytrophic tissues may lead to cellular and vascular proliferation resulting e.g. in blindness caused by obstruction [14, 26].

One of the factors governing tissue oxidation is the activity of superoxide dismutase (SOD) and registration of its concentration by chemical or biophysical methods may cast light on the intensity of tissue auto-oxidation. More precise knowledge in this field may facilitate the elaboration of effective therapeutic measures against the cellular damage caused by radical reactions.

SOD is a metalloprotein enzyme, its physiological role is to govern the reaction

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

in other words, inactivation of the superoxide anion [8, 9, 19].

D-penicillamine (DPA) is an important drug in the therapy of rheumatic arthritis. Later it was shown to decrease the elevated bilirubin level of jaundiced newborn babies [4, 15]. The complex of DPA and copper exerts an effect similar to that of SOD: it inactivates the toxic oxigen radicals [17, 27].

Proteolysis in pulmonary tissue is enhanced by the free radicals arising during the reduction of molecular oxygen [10, 22]. To counteract this damage, some defensive mechanisms are activated, viz. a mobilization of oxidable substrates [24], and an increase in SOD activity for dismutation of the toxic radicals [5].

In an earlier study we have shown that changes in SOD activity are a good marker of tissue auto-oxidation; in the present work we performed measurements of the malonyldialdehyde concentration for the same purpose and, in addition to DPA, the effects of a synthetic and of a natural antioxidant, 6,6'-methylene-bis/ /2,2,4-trimethyl-1,2-dihydroquinoline (MTDQ) and vitamin E [3, 14].

Materials and Methods

The studies were performed in 3—4 days old Wistar rats. The newborn animals were allotted to 16 groups.

Group 1: 16 animals kept in atmospheric air.

Group 2: 12 animals, in 95% oxygen environment. Figure 2 shows the time of administration of oxygen and the drugs.

Group 3: 12 animals, receiving daily 400 mg/kg MTDQ for three days.

Group 4: 12 animals, receiving MTDQ and kept in oxygen rich environment for three days.

Group 5: 8 animals received a dose of 1 g/kg of intraperitoneal DPA following a time-table similar to that used in animals treated with MTDQ.

Group 6: 8 animals given DPA treatment in oxygen rich environment.

Group 7: 9 animals were treated with intraperitoneal doses of 45 mg/kg vitamin E daily over three consecutive days.

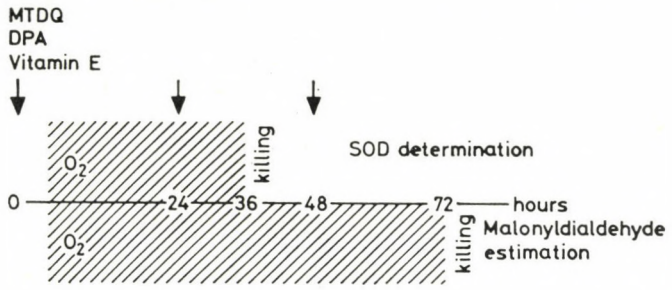

FIG. 2 Time-table of treatment of the experimental animals

Group 8: 7 animals received vitamin E, and were kept in 95% oxygen.

Groups 9 to 16 were corresponding controls.

The animals were killed by exsanguination. Malonyldialdehyde concentration and SOD activity were measured in liver and lung homogenates. The results were calculated for 1 g tissue. The method of SOD activity measurement has been described earlier (8, 14, 18, 19).

The concentration of malonylaldehyde was measured as follows. The tissue particles were homogenized in eightfold quantity of buffer, incubated at 37°C for 1.5 hours, then 100 μl trichloroacetic acid was added to 200 μl suspension, this was followed by centrifugation at 2000 g for 10 minutes. To 150 μl supernatant 100 μl thiobarbituric acid was added then kept in a boiling water bath for 10 minutes after cooling, and photometry at 532 nm was carried out. The results were calculated by aid of a calibration curve and expressed in mol/g wet tissue.

The results were analysed by Student's two-tailed t test.

RESULTS

Figure 3 shows the malonylaldehyde content of the lung tissue of newborn rats. The mean value for the control group kept in atmospheric air was 2.96×10^{-8} mol/g. Prolonged oxygenation markedly increased this value, by about 60% ($p < 0.001$). MTDQ caused a decrease of the tissue malonyldialdehyde level in animals

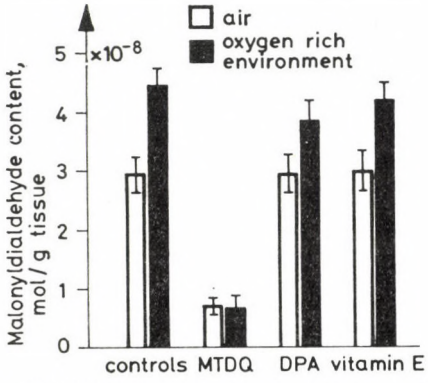

FIG. 3 Pulmonary malonyldialdehyde content of newborn animals kept in air and high oxygen environment, and treated with MTDQ, and vitamin E

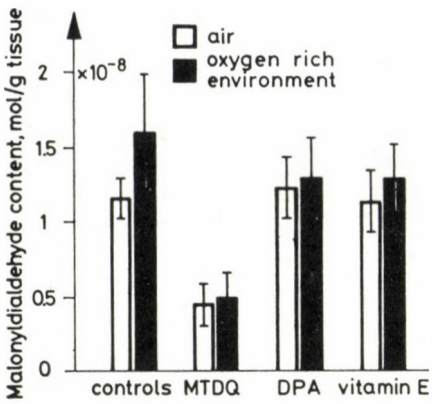

FIG. 4 Liver malonyldialdehyde content of newborn rats kept in air and high oxygen environment, and treated with MTDQ, DPA and vitamin E

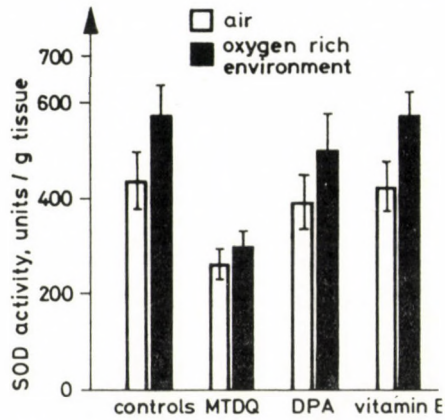

FIG. 5 Lung SOD actvity of newborn rats kept in air or oxygen rich environment, and treated with MTDQ, DPA and vitamin E

kept in both air and oxygen-rich environment, but the mean of these groups did not significantly differ from each other: 7.35 and 7.05×10^{-8} mol/g, respectively. This was a reduction by 75% compared to the value of the control group and by 85% if compared with the controls kept in oxygen rich environment. These differences were strongly significant ($p < 0.001$). DPA caused a less pronounced change; an insignificant decrease could only be shown in animals kept at high oxygen concentration ($p < 0.001$). Vitamin E had no demonstrable effect under either environmental condition. The most pronounced changes were caused by MTDQ, which significantly superior to the other antioxidants ($p < 0.001$).

Hepatic malonyldialdehyde concentrations reflected similar changes (Fig. 4). MTDQ elicited a significant decrease in either type of ambient

oxygen concentration, e.g. in animals kept in high oxygen the mean value dropped from 1.72×10^{-8} mol/g to 4.98×10^{-9} mol/g, corresponding to a reduction by 83% ($p < 0.001$). The two other antioxidants had an insignificant effect.

Figure 5 illustrates the SOD activity values in the lung. Oxygen alone increased the basal value of 436 U/g to 571 U/g ($p < 0.001$). MTDQ treatment decreased the mean value for the normal control group to 264 U/g, and that by 53% for the animals in oxygen rich environment ($p < 0.001$). DPA too, caused a decrease, in air by 10%, in high oxygen by 13% ($p < 0.001$). Vitamin E did not affect SOD activity. The effect of MTDQ was highly significantly stronger ($p < < 0.001$), than that of the two other drugs, independently of the oxygen concentration of ambient air.

Discussion

As the results showed, malonyldialdehyde concentration is a sensitive marker of the presence of free radicals liberated under the effect of various concentrations of oxygen. Protracted oxygenation led to a significant increase in the control animals, indicative of an accumulation of free radicals.

Oxygen induced a significant increase in liver and lung SOD activity. MTDQ caused a reduction by 50%; this suggests that the compound may play a role in counteracting the effect of free radicals. DPA caused a 10% reduction which too may be of use, in therapy.

Both MTDQ and DPA have been applied in treatment and we suggest to test their preventive effect against the side-effects of oxygen therapy in premature babies, especially retrolental fibroplasia and pulmonary dysplasia.

Malonyldialdehyde concentration as a marker of the quantity of peroxides liberated in the course of auto-oxidation was chosen by us since we had opportunity to compare this method with the common iodometric and polarometric assays [11, 16, 23, 25]. We have been able to show that the malonyldialdehyde method is most reliable and well-reproducible. We believe that the results can readily be transferred to the therapy of life-threatening conditions caused by elevated levels of free radicals in premature babies.

Acknowledgement

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G Balla MD
Pf. 32
H-4012 Debrecen, Hungary