RETINAL SUPEROXIDE DISMUTASE ACTIVITY IN NEWBORN KITTENS
EXPOSED TO NORMOBARIC HYPEROXIA
EFFECT OF VITAMIN E

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SUMMARY

Total superoxide dismutase activity (SOD) was measured in the retinas obtained from 18 kittens aged 1 to 11 days born to 6 litters. Pretreatment with 100 mg of α -tocopherol acetate S.C. followed by a 72 hr exposure to 80 % oxygen led to a higher ($P < .05$) SOD activity than in untreated animals. Retinas obtained from littermates who were not exposed to oxygen exhibited a greater ($P < .01$) SOD activity than those of the two hyperoxic groups.

These data suggest that normobaric hyperoxia leads to a fall in SOD activity which can be partially prevented by pretreatment with a large dose of vitamin E.

SPECULATION

The retina of the newborn kitten and of the premature human infant are reported to be equally vulnerable to hyperoxia. The development of lesions may be related to a fall in retinal SOD activity. The observation that vitamin E administration reduced this drop suggests an explanation for the partial protection it provides against retrolental fibroplasia.

INTRODUCTION

Hyperoxia enhances the formation of oxygen radicals in tissues and is thereby potentially toxic (16). The tolerance to hyperoxia is related to an increase in the activity of several enzymes (13). One of the most important seems to be the superoxide dismutase (EC 1-15-1) (SOD), involved in the elimination of the superoxide radical $O_2^{\cdot -}$, the first free radical generated. SOD catalyses the reaction $2 H^{\cdot} + 2 O_2^{\cdot -} \rightarrow O_2 + H_2O_2$ (16,25) and is essential to all aerobic organisms (17, 44). Prolonged exposure to oxygen concentrations greater than 90 % is fatal to organisms with a low SOD activity (8,18).

Premature infants treated with O_2 may develop both pulmonary complications (bronchopulmonary dysplasia, BPD) (34), and retinal damage (retrolental fibroplasia, RLF) (2,35). The appearance of pulmonary lesions has previously been shown to correlate with the absence of any rise in SOD activity during O_2 exposure (5,14,44). Vitamin E protects membranes from lipid peroxidation during hyperoxia, (11, 26,28,41,43) ; its administration to premature infants may decrease the frequency and the severity of RLF (22,36). Vascularisation of the retina in the newborn kitten is immature and developmentally similar to that of a human fetus at 28 weeks of gestation (3). Early retinal lesions induced by hyperoxia are known to progress in a similar pattern as those observed in premature human babies with oxygen retinopathy. The vulnerability of the retina to oxygen disappears after the 3rd week of life (4). Therefore the newborn kitten is a suitable experimental model to study the acute proliferative stages of RLF (3-4-36). We used it to further investigate biochemical events induced by hyperoxia in immature retina using SOD as an index of cell response. In addition, we looked at the effect of α -tocopherol on this response.

METHODS

Mongrel kittens weighing ($\bar{X} \pm SD$) 137 $\pm$ 56 g born in 6 litters of 3 kittens each were studied between 1 and 11 days of life. They were gavage fed every 6 hours a milk base formula* which provided low amounts of vitamin E (0.05 to 0.1 mg/day). The 3 kittens from each litter were randomly allocated to one of 3 groups : Group 1 consisted of 6 control animals kept in open cages and exposed to room air while the two study groups of 6 animals were maintained in closed plastic cages and exposed to 80 % O_2 for 3 days. As opposed to group 2 the animals from group 3 were given a S.C. injection of 100 mg of α -tocopherol acetate 12 hr prior to O_2 exposure. The ambient temperature was kept at 25°C. The O_2 concentration was continuously regulated between 80 and 82 % with a paramagnetic analyzer (BECKMAN OM 10) coupled to a system regulating the O_2 input. The CO_2 concentration was continuously measured (ONERA La Centrale de Chauffe, France). A servo-controlled system allowed the introduction of ambient air whenever CO_2 concentration exceeded 1 %.

At the end of the period of hyperoxia, the animals were anesthetized with an overdose of pentobarbital. Intracardiac blood was drawn for the assay of α -tocopherol (αT) by high pressure liquid chromatography and 10 ml of warm normal saline were injected *Neocarnis, Mixol Laboratories, France. through a catheter placed in the ascending aorta for washout of the eyes which were immediately removed. The retina was dissected removed and stored at -80°C. The period of time between removal of the eyes and deep freezing of the retinas never exceeded 15 minutes. Retinas were suspended in phosphate buffer (pH 7.2) homogenized for 30 seconds (Polytron homogeniser, kinematica, Lucerne) and centrifuged at 10 000 r.p.m. (Heraeus Christ Universal Junior 1 KS Model centrifuge for 20' at 0°C. Total SOD activity was then measured in the supernatant by the technique of Misra and Fridovich (30). Variance of the method as assessed on 5 duplicates was below 10 %. Results were expressed in units SOD per gram of fresh retina (U. SOD). A one way analysis of variance (38) was used to compare the three groups.

RESULTS

Large individual variations of SOD activity ($\bar{X} \pm SD$) were found (479 $\pm$ 133 U. SOD) in controls and, to a lesser extent, in the two hyperoxic groups. Levels of 315 $\pm$ 52 were recorded in group 2 and 390 $\pm$ 78 in group 3. Age of the animals at the time of the study did not appear to influence SOD activity. Within each litter O_2 exposure was consistently associated with a lower retinal SOD activity (fig). Hyperoxia led to a lower ($p < .01$) level of SOD in group 2 than in group 1. Pretreatment with vitamin E (group 3) partially prevented this drop. Kittens given vitamin E before O_2 exposure had retinal SOD levels greater ($p < .05$) but still lower than control littermates ($p < .01$) (table). The difference in SOD levels between littermates in group 2 and 3 was not related to post natal age, nor to the dose of vitamin E given per unit of body weight. There was no difference between group 1 and in group 2 for plasma αT (0.12 $\pm$ 0.1 vs 0.11 $\pm$ 0.08 mg/dl) which was 60-fold lower than the level (6.26 $\pm$ 2.53) in group 3 pretreated with vitamin E.

DISCUSSION

SOD is a ubiquitous enzyme which protects cellular metabolism by scavenging superoxide radical ($O_2^{\cdot -}$) (16,25). Hyperoxia usually produces an increase in SOD tissue activity, particularly in brain (8), lung (8, 23, 40), erythrocytes (6) and leucocytes (32, 37, 39). In young animals this elevation is greater and appears more rapidly than in adults (15, 40, 44).

Increased levels of SOD are needed for a good tolerance to hyperoxia. In rats submitted to hyperoxia, survival is directly related to pulmonary SOD levels (8). In infants with hyaline membrane disease (HMD) erythrocyte SOD levels increase during the second day when the subsequent clinical course is favorable but fall in those who eventually die or develop BPD (6). In premature infants who have died of HMD, pulmonary SOD levels have been found to be similar to those of infants dying from non pulmonary disorders (5) despite the fact that the former had been treated with O_2 . At high inspired O_2 concentrations, the absence of an increase in SOD activity is always associated with a poor outcome. We feel that a similar association would exist in other organs. Hyperoxia is an important factor in the development of RLF as well as of BPD.

Although both SOD (9, 19) and other enzymes (catalase : EC 1.11.1.6, GSH peroxidase : EC 1.11.1.9) (7) concerned with oxygen detoxification have been measured in various ocular tissues, including the retina, the reported studies have all been carried out on adult animals and have not examined the variations induced by hyperoxia. It is still not known if the normal retinal response to hyperoxia, particularly in adult animals, is an increase in SOD activity such as the one which occurs in lungs. However the adult retina, like other tissues in which this increase occurs, is not injured by hyperoxia (4). In this study all the kittens were less than 14 days old and therefore certainly susceptible to retinal vascular damage from hyperoxia. The results suggest a relationship in the newborn retina between a decreased SOD activity and hyperoxia induced lesions as it has been shown, in other tissues. The fall in SOD activity seems to reflect an inadequacy of tissue defense against hyperoxia and the increase in free radicals concentration which is related to it. Two possible mechanisms might be evoked to explain this fall in SOD activity.

1 - A disturbance of intracellular metabolism and an inhibition of O_2 detoxifying enzymes by their substrates as previously suggested by Rister (37). It is known that high concentration of H_2O_2 can inhibit SOD activity (29).

2 - Alterations of cell membranes through lipid peroxidation by $O_2^{\cdot -}$ (21, 27). These morphological changes are accompanied by a fall in metabolic activity and liberation of membrane bound enzymes (33).

The diminution in polyunsaturated fatty acid levels in endothelial cell membranes could also alter the synthesis of prostaglandins which control vasomotor tone, and thereby lead to the vaso-contraction initially seen in the retinas of premature infants. The biological properties of vitamin E support this second hypothesis. Vitamin E acts locally on cell membranes since it is incorporated into lipid structures (26). It has antioxidant activity (1, 41), decrease membrane lipid peroxidation (41) and membrane permeability (26). It prevents the liberation of membrane bound enzymes (11), such as phospholipase A_2 whose activity initiates prostaglandin synthesis. This could explain the inhibitory effect of vitamin E on prostaglandin synthesis (20). It is able to scavenge oxygen radicals as they penetrate into the cell and plays a role in its metabolic regulation (31).

Lesions observed in RLF as in BPD (24) are endothelial ; it suggests another comparison between them, since vitamin E deficiency favors interstitial oedema (42). Vitamin E has been found in the retina of mammals (10). Since vitamin E reserves are low in the premature (12) it is likely that retinal concentrations are also affected.

Vitamin E did not completely suppress SOD fall induced by hyperoxia. In a clinical study of oxygen treated premature infants, vitamin E administration was associated with a significant decrease in the incidence of retinal complications which however were not entirely abolished (22) ; the total dose of vitamin E of about 250 mg/kg body weight was given over several days. It could be assumed that effective levels have been achieved to late to prevent early injury ; however in our study kittens were given vitamin E 12 hours prior to hyperoxia and the dose was higher (500 - 1 000 mg/kg). It is tempting to suggest a relationship between the partial protective effect of vitamin E on the fall in retinal SOD levels and the incomplete protection observed for RLF. The present protocol was similar to that used by Phelps (36) who observed only a diminution in the incidence and severity of retinal vascular lesions. Vitamin E seems to improve the tolerance of the immature retina to hyperoxia but is not able to give complete protection. The protective effect of vitamin E which depends not only on its absolute concentration but also on the ratio of vitamin E to membrane lipids (28, 43) and an eventual uneven dis-

tribution in the body could explain the fact that it is only relatively effective even when given in high dosage.

The impairment of cellular defense against hyperoxia may be involved in the pathogenesis of RLF. Normobaric hyperoxia led to a fall in SOD activity in the retina of newborn kittens. As vitamin E reduced this fall, it is possible that vitamin E could provide a partial protection against oxygen retinopathy.

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Figure : Influence of hyperoxia on retinal superoxide dismutase (SOD) expressed in Units/g of wet tissue. This plot groups on the same vertical axis SOD activities of the 3 animals from each litter arranged from left to right in order of decreasing values.

table : Retinal Superoxide Dismutase Activity ($\bar{X} \pm SD$)

Groups	n	U/g retina
1	6	479 $\pm$ 113 (1)
2	6	315 $\pm$ 52 (2)
3	6	390 $\pm$ 78

(1) $p < .01$ between groups 1 and 2 and 1 and 3

(2) $p < .05$ between groups 2 and 3

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