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DISORDER IN HUMAN MYELIN INDUCED BY SUPEROXIDE RADICAL: AN IN VITRO INVESTIGATION

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SUMMARY: Potassium superoxide ($KO_2^{\cdot}$), applied as a source of superoxide radical directly *in vitro* to white matter from young adult human brain, caused the lipid phase of the myelin to change from a crystalline (ordered) state to a liquid crystalline (disordered) state. The myelin transition temperature decreased from 65°C to 37°C. This alteration was accompanied by a dramatic increase in the levels of lipid peroxidation products - malondialdehyde, a conjugated diene, and ethane.

These changes in human myelin, induced by direct application of $O_2^{\overline{\cdot}}$ radical, simulated myelin deterioration that occurs in the course of natural aging, thus, providing further substantiation for the notion that $O_2^{\overline{\cdot}}$ might be a major toxic agent associated with the aging process.

INTRODUCTION: In recent years, there has been increased interest in the involvement of $O_2^{\overline{\cdot}}$ in the initiation of tissue injury. This free-radical is generated by redox compounds such as quinones (1-3), nitro-compounds (4), dipyridylium compounds (5) and derivatives of phenazine (6), phenothiazine (1) and pteridine (7), which by their participation in cyclic reduction/autoxidation reactions, bring about the one electron reduction of molecular oxygen. The toxicity of $O_2^{\overline{\cdot}}$ and of the other active oxygen species (hydroxyl radical, singlet molecular oxygen) which. may be derived from it (8) has been demonstrated in diverse biological systems and appears to be responsible for a variety of pathological changes in animals (9-11).

The peroxidation of endogenous phospholipids, especially those in biological membranes, has long been thought to be the basis for a variety of toxic effects induced by free

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radicals (12). The evidence on which this concept is based involves measurements of the breakdown products of lipid hydroperoxides in biological systems exposed to toxicants or undergoing physiological stress (13). These breakdown products include malondialdehyde and its fluorescent conjugates as well as short-chain volatile hydrocarbons such as ethylene, ethane and pentane (14).

Lipid peroxidation is generally recognized to be a factor that contributes to cell and tissue deterioration during aging (15-17). In the case of human myelin, it has been previously demonstrated that age related destabilization of myelin lipid is associated with increased levels of lipid peroxidation (18). Superoxide radical is known to induce lipid peroxidation, and in the present study, we used $KO_2^{\cdot}$ (a source of superoxide radical) applied _in vitro_ directly to brain white matter to investigate the possible role of $O_2^{\overline{\cdot}}$ in the induction of myelin deterioration.

MATERIALS AND METHODS

Myelin Isolation.

The brains of 3 patients who died from accidental causes were obtained within 4-8 h after death. Each brain was examined by a neuropathologist to rule out central nervous system pathology. The cortex was removed, and the white matter was separated from the grey matter. Myelin was prepared as described earlier (18).

Application of $KO_2^{\cdot}$.

Finely powdered $KO_2^{\cdot}$ was mixed with 1 gm of central nervous system white matter in a ratio of 10% (w/w). After incubation for 10 min at $4^{o}C$, the reaction was terminated by the addition of 10 ml of $NaHCO_3$ (0.05M pH 7.5). The white matter was washed three times with this $NaHCO_3$ buffer prior to isolation of the myelin. Control samples were processed simultaneously with treated tissue, following an identical procedure except that $KO_2^{\cdot}$ powder was omitted.

X-Ray Diffraction of Myelin.

Myelin samples for X-ray diffraction were prepared as described previously (18). Wide angle X-ray diffraction patterns were recorded at various temperatures using CuKα radiation from a point-focussed X-ray tube (type PW 2103/01) on a Philips (type 1030) camera under conditions in which the samples retained 50-75% moisture with respect to final dry wt. The lipid phase transition temperature, defined as the highest temperature at which gel phase lipid can be detected, was determined to within 1°C.

Determination of Lipid Peroxidation Products.

(1) Malondialdehyde

Levels of malondialdehyde (MDA), a breakdown product of unsaturated fatty acid hydroperoxides, were measured in the white matter homogenates using a modified thiobarbituric acid (TBA) test (19). MDA levels were calculated relative to a standard preparation from the hydrolysis of 1,1,3,3,-tetramethoxy-propane (20).

(2) Conjugated Diene

Conjugated dienes, which are formed during the peroxidation of unsaturated fatty acids, exhibit spectra characterized by an intense absorption at 233 nm and were estimated by the method of Buege and Aust (21).

(3) Ethane Measurement

Levels of ethane produced in the white matter of human brain were measured immediately before and after treatment with $KO_2^{\bullet}$. Finely powdered $KO_2^{\bullet}$ was mixed with 1 mg of white matter at a concentration of 10% (w/w) in a 6 ml test tube, which was immediately sealed with a rubber cap. A 1 ml gas sample was withdrawn from the test tube at times 0, and 10 min, and analyzed for ethane under isothermal conditions (65°C) with a Perkin-Elmer series 900 gas chromatograph fitted with an AlO_3 column and a flame-ionization detector. Control samples were treated and analyzed in the same way except that $KO_2^{\bullet}$ was omitted.

RESULTS AND DISCUSSION

Wide angle X-ray diffraction has demonstrated that direct treatment of white matter with superoxide radical in the form of $KO_2^{\bullet}$ induces disorder in myelin of the adult human central nervous system. Diffraction patterns were recorded at 40°C, which is below the lipid phase transition temperature of control myelin preparations. Patterns for untreated myelin featured two lipid reflections, a sharp reflection at a Bragg spacing of 4.15 Å and a broad reflection at centered at 4.6 Å (Fig. 1A). The sharp 4.15 Å reflection represents gel phase lipid and the broad 4.6 Å reflection is derived from liquid crystalline lipid (22). Thus, at 40°C, normal myelin contains lipid in both gel (ordered) and fluid (disordered) states. For myelin treated with $KO_2^{\bullet}$, the only lipid reflection detectable in X-ray diffraction patterns was the broad diffuse band centered at a Bragg spacing

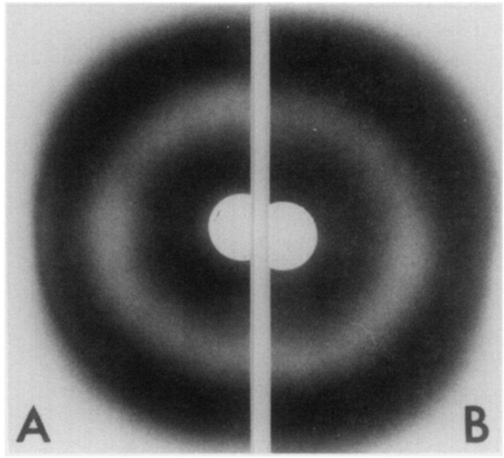

Fig. 1. Wide-angle X-ray diffraction patterns for isolated myelin fractions from human brain. Patterns were recorded at 40°C. (A) Pattern from untreated normal myelin featuring (from outside to inside) a sharp band centered at a Bragg spacing of 4.15Å and two broad diffuse bands centered at Bragg spacings of 4.6 and about 10 Å. (B) Pattern for myelin isolated from white matter of normal adult brain treated with $KO_2^{\cdot}$ for 10 min featuring (from outside to inside) two broad bands centered at Bragg spacings of 4.6 and 10 Å.

of 4.6 Å (Fig. 1B), indicating that the myelin lipid changed from a mixture of two types of lipid into exclusively liquid-crystalline phase.

The myelin transition temperature, which is operationally defined as the highest temperature at which gel phase lipid can be detected, reflects the composition of the phospholipids and, in particular fatty acids, contributing to the gel phase. For normal human adult myelin, the transition temperature of the lipid phase was 65°C (Table 1). Following $KO_2^{\cdot}$ treatment, however, the myelin transition temperature decreased by about 28°C, indicating that a portion of the lipid underwent a phase change from an ordered state to a disordered state. Thus, it appears that stability of the myelin bilayer was substantially reduced following direct exposure to $O_2^{\bar{\cdot}}$.

This reduction in bilayer stability coincides with the induction of lipid peroxidation in white matter treated with $KO_2^{\cdot}$. Levels of MDA, a product of lipid peroxidation, were 11 times

Table 1. Effects of $KO_2^{\cdot}$ Treatment on the Lipid Phase Transition Temperature of Adult Myelin and on Parameters of Lipid Peroxidation.

		Myelin Transition Temperature (°C)		Lipid Peroxidation Products: MDA nmole/gm fresh tissue		Conjugated Diene O.D./gm fresh tissue		Ethane ppm/gm fresh tissue
Untreated	$\bar{x}$	65.0	$\bar{x}$	6.47	$\bar{x}$	15.17	$\bar{x}$	0.33
	$S_{\bar{x}}$	0.0	$S_{\bar{x}}$	0.96	$S_{\bar{x}}$	0.57	$S_{\bar{x}}$	0.08
Treated	$\bar{x}$	37.0	$\bar{x}$	71.72	$\bar{x}$	21.50	$\bar{x}$	2.14
with $KO_2^{\cdot}$	$S_{\bar{x}}$	1.5	$S_{\bar{x}}$	4.42	$S_{\bar{x}}$	1.18	$S_{\bar{x}}$	0.19

$\bar{X}$ = Mean: $S_{\bar{x}}$ = Standard error of the mean.

higher in white matter treated with $KO_2^{\cdot}$ for 10 min than in control white matter (Table 1). Two other indices of peroxidation - ethane production and levels of conjugated dienes - were also significantly higher as a result of $KO_2^{\cdot}$ treatment (Table 1). It would appear, therefore, that $O_2^{\overline{\cdot}}$ reduces myelin stability through the induction of lipid peroxidation.

hese alterations in biophysical properties of human myelin in response to $O_2^{\overline{\cdot}}$ treatment similate changes that occur during the course of normal aging (18), although they are more dramatic. Consequently, this study provides additional evidence for the potential role of free radicals as important factors in the aging processes of the human brain.

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