Free Rad. Res. Comms., Vol. 1, No. 6, pp. 355-359
8755-0199/86/0106-0355 $25.00/0

Printed in Great Britain

ENHANCEMENT OF ENZYME-CATALYZED PRODUCTION OF REACTIVE OXYGEN SPECIES BY SUSPENSIONS OF "CROCIDOLITE" ASBESTOS FIBRES

ERICH F. ELSTNER, WOLFGANG SCHÜTZ and GEORG VOGL

Institute of Botany and Mikrobiology, Technical University of Munich, 8000 Munich 2, W. Germany

(Received November 1, 1985)

Ethylene release from methylthio-ketobutyric acid is an indicator for activated oxygen species of the $OH^{\cdot}$-radical type. Xanthine oxidase plus xanthine or diaphorase in the presence of NADH and juglone produce $OH^{\cdot}$-type oxy-radicals. The production of reactive oxygen species in these enzymatic systems is enhanced by "crocidolite" asbestos fibres.

Key words: Asbestos, oxygen activation, xanthine oxidase, NADH-diaphorase, lung diseases

INTRODUCTION

Prolonged inhalation of asbestos fibres of a certain size produces fibrosis of the lung ("asbestosis") as well as malignant tissue transformations[1]. Oxidative injury to airway epithelial cells induced by asbestos fibres was recently reported by Mossman and Landesman[2]. They observed that ^{75}Se-selenomethionine — labelled hamster trachel epithelial cells released radioactive material into the culture medium upon incubation with certain asbestos samples. The enzyme superoxide dismutase (SOD) inhibited asbestos — induced ^{75}Se release whereas catalase and the singlet oxygen quencher 1.4-diazabicyclooctane (DABCO), had no effect or were slightly stimulating. They concluded from their results that asbestos fibres enhanced the cellular production of deleterious superoxide radicals whereas H_2O_2 or singlet oxygen ($^1O_2$) are apparently not involved in cell damage.

New light into the understanding of asbestos — induced cell damage was brought by Weitzman and Gracefa[3] who showed that suspensions of different asbestos types upon incubation with H_2O_2 yielded "spin-trapped" electron spin resonance (ESR) signals strongly corresponding to hydroxyl radicals ($OH^{\cdot}$) and to a lesser extent to $O_2^{\cdot -}$. From the influence of certain iron chelators on the production of these signals they concluded that asbestos was able to generate $OH^{\cdot}$ and $O_2^{\cdot -}$ from H_2O_2 via the catalysis of iron as an integral part of the asbestos complex.

The work presented here was undertaken to

a) investigate on enzymatic model reactions possibly bridging the gap between the experiments done with cell cultures[2] and those on the basis of "pure" physical chemistry[3], and

b) to find an explanation for the apparent discrepancy concerning the reactive oxygen species involved in this toxicity, e.g. the function of H_2O_2 reported in the above papers[2,3].

MATERIALS AND METHODS

NADH, KMB and SOD were obtained from Sigma, juglone and diaphorase from Serva and catalase and xanthine oxidase were from Boehringer-Mannheim. Crocidolite was obtained from the "Bayerisches Landesamt für Umweltschutz" Munich.

The enzymic systems tested in respect to enhancement of oxygen activation were

a) xanthine oxidase with xanthine as substrate and

b) diaphorase (from *Clostridium kluyveri*) with NADH as electron donor.

The reaction mixtures were incubated for 30 min in 14 ml Fernbach flasks fitted with gas — tight serum rubber stoppers. 1 ml gas from the head space was withdrawn with gas-tight syringes after the incubations and analysed by gas chromatography[4].

TABLE I
Stimulation by crocidolite and inhibition by catalase and SOD of xanthine oxidase — driven ethylene release from KMB

Complete system: Phosphate buffer, xanthine oxidase, xanthine; for details see materials and methods	
conditions	ethylene formed (pmol/30 min)
complete	3420
− xanthine oxidase	0
− xanthine	0
− xanthine − xanthine oxidase + crocidolite	12
complete + 5 U SOD	840
complete + 50 U catalase	760
complete + 0.5 mg crocidolite	7636

The reaction mixtures were as follows:

1) xanthine oxidase system: 0.1 M phosphate buffer pH 7.9 (chelex — treated): xanthine, 0.5 μmol; xanthine oxidase, 0.5 U; KMB, 5 μmol; 5 μmol and crocidolite, 0.5 mg in a total volume of 2 ml.

2) diaphorase system: 0.1 M phosphate buffer pH 7.9 (chelex treated): NADH, 1.5 μmol; diaphorase, 2.2 U; KMB, 2.5 μmol; juglone, 10 nmol and crocidolite, 0.2 mg in a total volume of 2 ml.

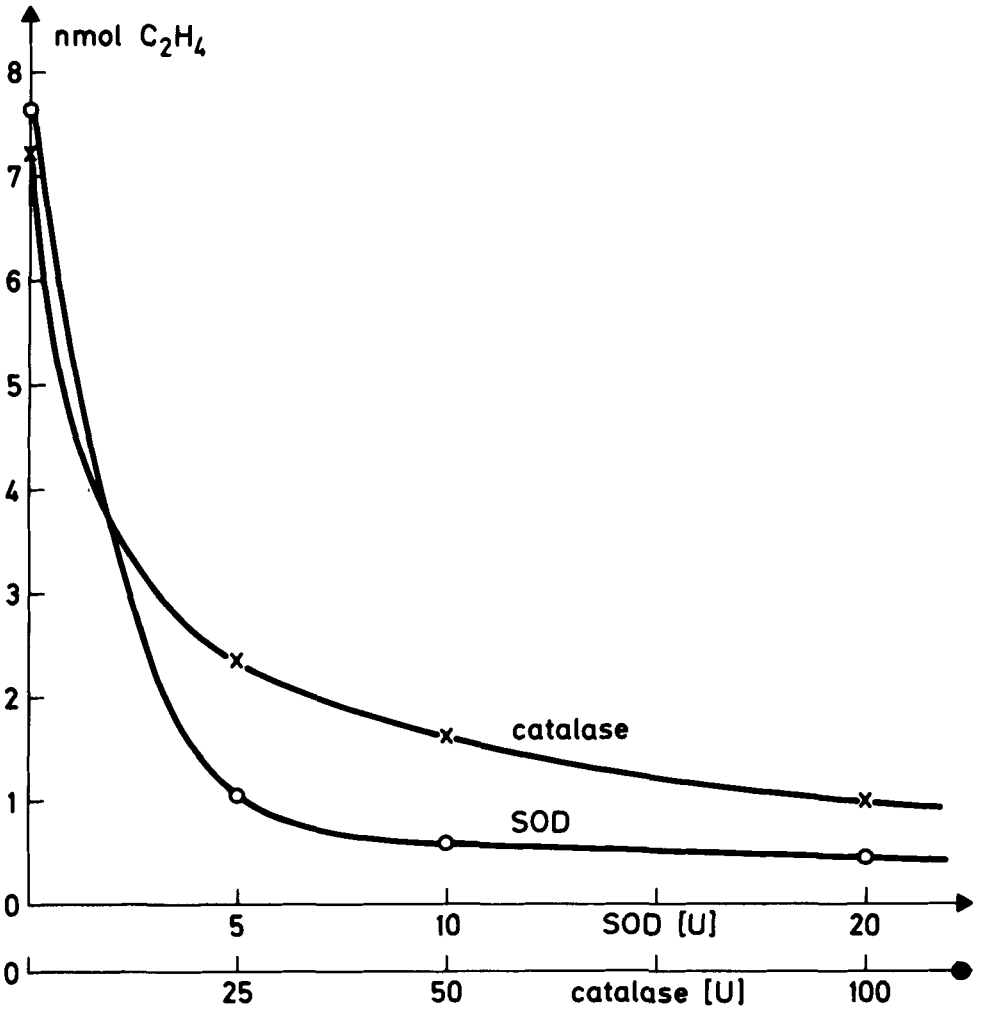

FIGURE 1 *Inhibition by SOD and catalase of crocidolite* — stimulated ethylene release from KMB driven by xanthine-xanthine oxidase.

Reaction conditions were identical to those in table I and as described under "material and methods" for 1) xanthine oxidase.

RESULTS AND DISCUSSION

As shown in Table I, xanthine oxidase- driven KMB oxidation releasing ethylene, is inhibited to approximately the same extent by 5 U SOD or 50 U catalase. Addition of 0.5 mg of crocidolite by ca. 100% stimulates ethylene formation, indicating an enhancement of the "Haber-Weiss" — type reaction[5]. This crocidolite stimulated KMB fragmentation is under the control of both SOD and catalase (Fig. 1).

NADH diaphorase also catalyzes KMB fragmentation releasing ethylene. This reaction is strongly stimulated by certain quinones such as juglone and to some extent also by crocidolite. These reactions are inhibited by ca. 80–90% by 65 U catalase. SOD (50 U) in the absence of crocidolite inhibits by ca. 50 %. In the presence of crocidolite, however, SOD only inhibits by ca. 25%.

If we assume that asbestos fibres in lung tissue are "microcenters" of inflammation brought about by partial engulfment by macrophages[1], stimulation of production of $OH^{\cdot}$ — like oxidants (Table I) or loss of part of the SOD control (Table II) might contribute to the disease symptoms such as inflammations, fibrous scarring or cancer, significantly increased in certain populations of asbestos workers. Independent from studies on asbestos toxicity[1], it has been postulated that activated oxygen and/or loss of control by defence mechanisms may lead to spontaneous cancer[6]. Furthermore, cooperative effects of asbestos fibres and other air pollutants have been discussed[7], but no conclusive data have been presented so far. Overadditive effects of

TABLE II

Effect of juglone, crocidolite, SOD and catalase on NADH-diaphorase — driven ethylene formation from KMB

Complete system: Phosphat buffer, NADH, diaphorase for details see materials and methods

conditions	ethylene formed (pmol/30 min)	+ 50 U SOD	% inhibition	+ 65 U catalase	% inhibition
complete	150	75	50	25	80
complete + crocidolite	230	45	80	28	85
complete + juglone	1970	920	53	420	80
complete + juglone + crocidolite	2400	1750	25	280	87
complete + NADH	10[a]				
complete − diaphorase	0				
complete − NADH, − diaphorase + juglone	25[a]				
complete — NADH, − diaphorase + 0.2 mg crocidolite	0				

[a] values obtained in laboratory "daylight". In the absolute dark these reactions were essentially zero, the complete reaction by ca. 20% reduced. This effect is due to the photodynamic activity of juglone and the flavin moiety in diaphorase.

"crocidolite" asbestos fibres and HSO_3^- on oxygen activation and concomitant changes in the respective reaction mechanisms will be discussed in detail in a forthcoming report (in preparation).

Acknowledgements

This work was financially supported by "SHELL" Int. Petroleum Maatschapij B.V., Den Haag, Netherlands. We wish to thank Dipl. Phys. K. Coy, Bayerisches Landesamt für Umweltschutz, for stimulating discussions and for the sample of crocidolite.

References

1. B. Mossman, W. Light and E. Wei, *Ann. Rev. Pharmacol., Toxicol.*, **23**, 595–615, (1983).
2. B. Mossman and J.M. Landesman, *Chest*, **83**, 50s–51s, (1983).
3. S.A. Weitzman and P. Gracefa, *Arch. Biochem. Biophys.*, **228**, 373–376, (1984).
4. R.J. Youngman, G.R. Wagner, F.W. Kühne and E.F. Elstner, *Z. Naturforsch.*, **40c**, 409–414, (1985).
5. G. Cohen, in *Superoxide and Superoxide Dismutases eds.* A.M. Michelson, J.M. Mc Cord and I. Fridovich, (Academic Press 1977) pp. 317–321.
6. J.R. Totter, *Proc. Natl. Acad. Sci. USA*, **77**, 1763–1767, (1980).
7. I.J. Selikoff and D.H.K. Lee, (eds.) *Asbestos and Disease* (Academic Press, New York, 1978).

Accepted by Dr. J.V. Bannister