INHIBITION OF SUPEROXIDE ANION PRODUCTION IN GUINEA PIG POLYMORPHONUCLEAR LEUKOCYTES BY A SELENO-ORGANIC COMPOUND, EBSELEN*

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Production of superoxide anion (O_2^-) induced by tetradecanoyl phorbol acetate (TPA) in intact guinea pig polymorphonuclear leukocytes (PMNL) was markedly inhibited by a seleno-organic compound, 2-phenyl-1,2-benzisoselenazol-3(2*H*)-one (Ebselen), with glutathione peroxidase-like activity. The compound almost completely inhibited O_2^- production by a particulate fraction prepared from TPA-treated PMNL at a concentration as low as 250 nM.

Keywords — polymorphonuclear leukocytes; superoxide anion production; seleno-organic compound; anti-inflammatory action; glutathione metabolism

INTRODUCTION

Production of active oxygen metabolites including superoxide anion (O_2^-) is induced by phagocytosis as well as by treatment with various membrane perturbants in polymorphonuclear leukocytes (PMNL).¹⁾ Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in cell membrane is involved in the production of O_2^- , a primary product among the active oxygens.²⁾ We have interested in the mechanism for the induction of O_2^- production and regulation of NADPH oxidase activity in guinea pig PMNL.^{3–7)} The active oxygens are important in the bactericidal function of PMNL, but their excessive production is harmful not only to PMNL but also to surrounding tissues and causes pathological changes such as inflammatory reactions.⁸⁾ Thus, it is desirable that O_2^- production is controlled at an appropriate level.

A seleno-organic compound, 2-phenyl-1,2-benzisoselenazol-3(2*H*)-one (Ebselen), has been reported to have glutathione peroxidase-like activity.^{9,10)} Since glutathione peroxidase, which also contains selenium, is related to NADPH metabolism, it is expected that Ebselen may have an effect on NADPH oxidase activity to change O_2^- production. We report here that the compound actually has such an effect at very

low concentration.

MATERIALS AND METHODS

The preparation of PMNL from the peritoneum of guinea pigs after injection of casein, that of the particulate fraction from PMNL, and the assay for O_2^- production on the basis of the reduction of ferricytochrome c, were all performed by the same procedures reported in our previous papers.^{3,5)}

RESULTS AND DISCUSSION

O_2^- production in intact PMNL incubated at 37 °C in Hanks'-Pipes (piperazine *N,N'*-bis[2-ethane sulfonic acid]) (pH 7.2) buffer containing 100 μ M cytochrome c, 10 U/ml catalase and 0.2 μ g/ml tetradecanoyl phorbol acetate (TPA), was completely inhibited by the addition of Ebselen at 10 μ M (Fig. 1). Since Ebselen was dissolved in dimethylsulfoxide (DMSO), the same amount of DMSO was added to the control. The O_2^- production was superoxide dismutase (SOD)-sensitive and was not observed in the resting PMNL.

To ascertain whether the effect of Ebselen was on NADPH oxidase or on other systems such as an NADPH-producing system, a particulate fraction with NADPH oxidase activity, a

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FIG. 1. Effects of Ebselen on TPA-induced O_2^- Production in Intact PMNL

Ebselen was added at the indicated concentrations to the incubation medium of either guinea pig PMNL in which O_2^- production was stimulated by coexisting $0.2 \mu\text{g/ml}$ TPA (open circle) or resting PMNL (closed circle). O_2^- production was measured as SOD-sensitive reduction of added ferricytochrome c. The O_2^- production by the TPA-treated PMNL was $5.2 \text{ nmol/min}/10^6$ cells, in the absence of Ebselen.

FIG. 2. Effect of Ebselen on O_2^- Production in the Particulate Fraction from TPA-treated PMNL

Ebselen was added at the indicated concentrations to the solution of the particulate fraction from either guinea pig PMNL which were treated with $0.2 \mu\text{g/ml}$ TPA for 5 min (open circle) or resting PMNL (closed circle). The measurement of O_2^- production was the same as in Fig. 1. The O_2^- production by the particulate fraction from the TPA-treated PMNL was $1.7 \text{ nmol/min/fraction}$ from 10^6 cells, in the absence of Ebselen.

$28000 \times g$ precipitate, was prepared from sonicates (2 A, 20 s) of PMNL treated with $0.2 \mu\text{g/ml}$ TPA for 3 min. O_2^- production by the preparation suspended in Hanks'-Pipes buffer containing $100 \mu\text{M}$ cytochrome c, 10 U/ml catalase and 0.5 mM NADPH was markedly inhibited by the addition of Ebselen at a concentration as low as 250 nM (Fig. 2). The reason for the difference in the effective concentration of Ebselen between intact PMNL system and the particulate fraction is not clear, but it may be due to the difference between the intact cell and cell-free system in the interaction of Ebselen with NADPH oxidase. Though the reason was not clear, the inhibition of NADPH oxidase by Ebselen in the cell free system was observed after a lag time of 4–6 min, whereas no such lag time was found in the case of intact PMNL. It is unlikely that the effect of Ebselen on the O_2^- production was due to the effect on cytochrome c in the assay system, since reduction of cytochrome c with xanthine oxidase system examined by a standard procedure,⁹⁾ was little affected by Ebselen up to $40 \mu\text{M}$ (data not shown).

The mechanism for the inhibitory effect of

Ebselen on O_2^- production is to be elucidated further. The compound may affect NADPH oxidase directly. A Lineweaver-Burk plot analysis of the inhibitory effect showed an uncompetitive type inhibition, suggesting that Ebselen may affect the enzyme-substrate complex (data not shown). This result may coincide with the above-mentioned lag time for the appearance of the inhibitory effect of the compound. Another possibility is that the compound causes a decrease in the reduced form of glutathione since the compound has glutathione peroxidase-like activity.^{10,11)} The reduced form is reproduced by the action of glutathione reductase which requires NADPH. Thus, the availability of NADPH as a substrate for NADPH oxidase may be altered by the presence of Ebselen. It has been reported that Ebselen also has inhibitory effects on macrophage function¹²⁾ and lipid peroxidation.¹³⁾ It is also known that selenium deficiency in relation to glutathione peroxidase activity is involved in arachidonate cascade.^{14–16)} These findings are all related to inflammatory responses. The inhibitory effect of Ebselen on O_2^- production in PMNL, observed here, may partic-

ipate in the anti-inflammatory action of Ebselen.

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