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RAPID COMMUNICATIONS

Inhibition by Ebselen of microsomal NADPH-cytochrome P450-reductase *in vitro* but not *in vivo*

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Introduction

Ebselen (PZ 51, 2-phenyl-1,2-benzisoselenazol-3-(2H) one) is a new selenium-containing anti-inflammatory agent which catalyzes a reaction analogous to the enzymatic reaction sequence of glutathione peroxidase (E.C. 1.11.1.9) (1,2). A peroxide-scavenging effect of ebselen was also shown with mouse macrophages (3). In isolated hepatocytes, an antioxidant function was ascribed to the compound when low-level chemiluminescence, alkane formation and malondialdehyde production were monitored (4). Recently, ebselen has been shown to inhibit leukocyte lipoxygenase activity (5).

At this stage of development the question arises whether the anti-inflammatory action of ebselen might be due to a specific interaction with enzymes involved in the synthesis or release of inflammatory mediators, or to a general antioxidant function, diminishing the " peroxide tone " (6). An essential prerequisite for studying antioxidant functions of the compound in model systems involving microsomal lipid peroxidation is to determine whether ebselen interferes with the microsomal electron transfer.

Here we report that ebselen inhibits *in-vitro* mouse liver microsomal NADPH-Cyt P450-reductase activity with an IC_{50} as low as 0.13 μM.

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Materials and Methods

Male NMRI mice fed on the Alma-diet R+M H1003 were housed in plastic cages with free access to food and tap-water. The animals were kept under environmentally controlled conditions. Microsomes were prepared according to (7). NADPH-Cyt P450-reductase was assayed according to (8) with either cytochrome c or ferricyanide as substrate. All other enzyme activities were determined as described in ref.8. Ebselen and related compounds were a gift of Dr. E. Graf, Nattermann, Cologne. Ebselen was dissolved in 0.3 moles/liter potassium phosphate buffer pH=7.7 by ultrasonication and then added to the microsomal suspensions in the same buffer. PZ 25, the sulfur analogue of ebselen, was dissolved in ethanol and then added to the microsomes to yield a final concentration of 2 % ethanol; the methyl-seleno derivative (2-methylselenobenzanilide) was dissolved in dimethylsulfoxide (DMSO) to yield 0.5 % DMSO in the reaction mixture. The corresponding control incubation contained the same amount of either ethanol or DMSO. All other compounds and enzymes used were analytical grade reagents obtained from Sigma.

Results and Discussion

The data in Fig.1 show that in the presence of ebselen, an instantaneous, irreversible and concentration-dependent inhibition of microsomal NADPH-cytochrome P450-reductase activity was observed. In a Dixon plot, the ebselen concentration needed to result in 50 % inhibition was extrapolated to 0.13 μM. The inhibition was not avoided by preincubation of the microsomes for 5 minutes with 200 μM NADPH or $NADP^+$. When untreated microsomes were combined in different proportions with ebselen-inhibitec microsomes, we found that the inhibition of NADPH-Cyt P450-reductase activity could not be diluted out. In a similar type of experiment as in Fig. 1, an IC_{50} of 3 μM was found for the sulfur analogue of ebselen, PZ 25, while the methyl-seleno derivative had essentially no inhibitory effect up to 30 μM. For comparative purposes, the known inhibition of NADPH-cyt P450-reductase activity by dithiobisnitrobenzoic acid (DTNB) (7) was also investigated in an analogous experiment; an IC_{50} of 150 μM was found for this compound under our conditions. In all these cases (ebselen, PZ 25 and DTNB) a similar type of concentration dependence and an apparently linear Dixon-plot was obtained. These observations indicate that the essential sulfhydryl group of the enzyme (9) is likely to be the target of these inhibitory compounds. The chemistry of ebselen and PZ 25 allows them to react easily with e.g. glutathione and other sulfhydryls to form mixed selenodisulfides or disulfides, respectively (2,10).

We were interested to determine whether this in-vitro inhibition has any bearing in-vivo. Therefore, a group of eight mice and the same number of controls were fed a diet containing 200 ppm ebselen for one week; this corresponds to an anti-inflammatory active dose (11). Then, microsomes were prepared and assayed for reductase activity. Essentially no significant difference in NADPH-Cyt P450-reductase activity, aminopyrine-N-demethylation, ethoxycoumarine-O-deethylation rate, cytochrome P450 and cytochrome b5 contents and glutathione-S-transferase activity was found between the ebselen-fed and the control group. Ex-vivo the inhibition of reductase activity was not detectable. One of the major reasons for this might be that the compound, in its original chemical form, may not reach the liver cell organelles, where the enzyme is localized, because it may react rapidly with cellular thiols and undergo biotransformation as well. In line with this interpretation, in-vivo treatment of mice with 500 mg/kg ebselen did not influence the extent of glutathione depletion caused by paracetamol or bromobenzene intoxication, i.e. the metabolism of these compounds was not blocked (12). Treatment with 500 mg/kg ebselen alone did not significantly alter liver glutathione levels. Similarly, paraquat toxicity in rats was not prevented by pretreatment with 200 mg/kg ebselen (13). These data indicate that in microsomal systems care must be taken in the interpretation of antioxidant effects of ebselen which may be attributable to a specific interference with the electron transfer from NADPH-cytochrome P450-reductase to cytochrome P450.

None the less, the ability to inhibit the NADPH dependent reductase activity provides a very useful and promising tool in order to study partial reactions of the monooxygenase in microsomal suspensions.

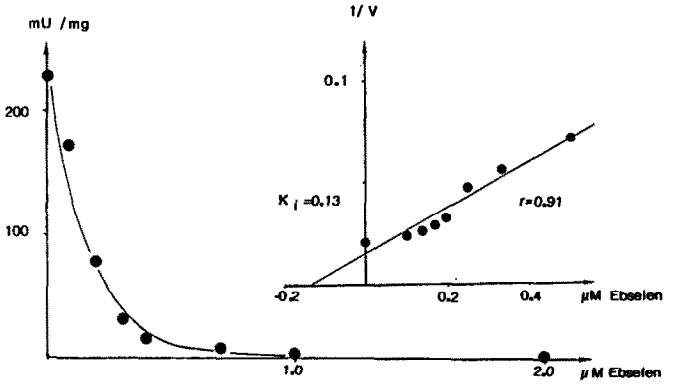

Figure 1:
Inhibition of mouse liver microsomal NADPH-cytochrome P450-reductase activity by ebselen
Conditions: 0.3 mol/liter potassium phosphate buffer, pH = 7.7, 37 °C, 6 µg/ml microsomal protein, total volume 1 ml
Inset: Dixon-plot

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