Biochemical Pharmacology, Vol. 38, No. 4, pp. 619–625, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

OPTICAL SPECTRAL STUDIES OF EBSELEN INTERACTION WITH CYTOCHROME P-450 OF RAT LIVER MICROSOMES

NIKOLAUS KÜHN-VELTEN and HELMUT SIES*

Institut für Physiologische Chemie I and II, Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf, Federal Republic of Germany

(*Received* 24 *June* 1988; *accepted* 16 *September* 1988)

Abstract—Interaction of ebselen, an anti-inflammatory compound of low toxicity, with rat liver cytochrome P-450 is used as a model system to quantify possible interactions of seleno-organic compounds with sulfhydryl groups of intracellular membrane-bound proteins. Ebselen induces a unique difference spectrum (maximum at 405 nm, minima at 385 and 425 nm) after addition to microsomes under *in vitro* conditions. This spectrum indicates an interaction with the thiolate anion at cytochrome P-450; it can be blocked by previous addition of dithioerythritol. With uninduced microsomes, addition of ebselen converts maximally 50% of the cytochrome P-450 to P-420 in a time-dependent (nearly complete effect within 10 min) and concentration-dependent manner (halfmaximal effect with 50 μM at 1 nmol/ml cytochrome P-450 concentration) *in vitro*. In phenobarbital- and 3-methylcholanthrene-induced microsomes, 73% and 64%, respectively, of cytochrome P-450 are converted to P-420 in presence of 200 μM ebselen. It is assumed that only certain isoenzymes of the total hepatic cytochrome P-450 are accessible to ebselen. Bovine serum albumin at physiological concentrations and sulfhydryl compounds such as dithioerythritol are effective in preventing this cytochrome P-450 inactivation by ebselen. Specificity studies reveal that variation of the N-substituent in the benzisoselenazolone system does not influence cytochrome P-450 inactivation, whereas ebselen derivatives with methylated or glucuronidated selenium moiety as well as diselenides do not convert cytochrome P-450 to P-420. It is concluded that benzisoselenazolones are able to interact with sulfhydryl groups of membrane-associated proteins *in vitro*.

Ebselen (PZ51; 2-phenyl-1,2-benzisoselenazol-3(2H)-one) represents a new class of anti-inflammatory agents. This seleno-organic compound does not release selenium from the molecule and therefore shows low toxicity in subacute animal experiments (see Ref. 1 for review). Ebselen exhibits GSH peroxidase-like activity [2–4] thus imitating the natural, selenium-dependent enzyme, glutathione-peroxidase (glutathione: hydrogen-peroxide oxidoreductase, EC 1.11.1.9), but accepting not only reduced glutathione (GSH) but also dithioerythritol [5] or *N*-acetylcysteine [6] as reductant. Ebselen is able to inhibit ADP-Fe-induced lipid peroxidation [5] and has been found to decrease diquat-induced cytotoxicity [6] in isolated hepatocytes.

During liver perfusion, ebselen is mainly metabolized to an Se-glucuronide, secreted in the bile, and to Se-methylated derivatives, secreted in the effluent perfusate [7]. Since, in addition, a 4′-hydroxylated metabolite (4′-hydroxy-2-methyl-selenobenzanilide) can be identified [7], the ebselen biotransformation process may include a hepatic cytochrome P-450-dependent mono-oxygenase system. A thiolate group ligated to the heme iron is essential for the catalytic function of cytochrome P-450 [8, 9]. Since benzisoselenazolones may interact with sulfhydryl groups provided by cysteinyl residues in proteins instead of the sulfhydryl groups of GSH or analogous compounds, we have characterized interactions of ebselen and of its derivatives with cytochrome P-450 in rat liver microsomes by measurement of optical difference spectra.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

Chemicals. Ebselen and its analogues and derivatives (see Table 3) were a kind gift from Dr. E. Graf (Rhône-Poulenc/Nattermann Co., Cologne Research Center, F.R.G.). All chemicals used were of analytical grade.

Preparation of microsomal suspensions. Livers from male Han:Wistar rats (250 g body wt, pentobarbital anesthesia) were perfused hemoglobin-free for 3 min without recirculation with a Krebs–Henseleit buffer medium. Livers were then homogenized in 250 mM sucrose, 10 mM Tris, pH 7.4 (TS buffer) using a Potter-Elvehjem homogenizer. Homogenates were centrifuged for 10 min at 750 *g* and 10 min at 8700 *g*. Supernatants from the last centrifugation step were subjected to a further centrifugation for 10 min at 8700 *g*. Supernatants were then centrifuged at 130,000 *g* for 45 min. The pellets were rehomogenized in TS buffer, and the ultracentrifugation step was repeated once more. The final pellet was rehomogenized in TS buffer to a final protein concentration of 40 mg/ml and stored at −80°. Protein concentrations in microsomal suspensions were determined by the Lowry method using bovine serum albumin as the standard [10]. In some experiments, liver microsomes were prepared

from animals which received either daily injections of phenobarbital (80 mg/kg body weight) over 4 days or a single injection of 3-methylcholanthrene (20 mg/kg body weight).

Spectral measurements. Difference spectra were recorded with a Shimadzu UV300 spectrophotometer in the single wavelength/double beam mode. Cytochrome P-450 concentrations were determined as described in [11] using a molar absorption coefficient of 91,000 l/(mol × cm) for the A_{450nm} minus A_{490nm} difference. For correct calculation of changes in cytochrome P-450 concentrations induced by ebselen and its analogues, CO/dithionite-reduced cytochrome P-450 minus dithionite-reduced cytochrome P-450 absorption differences were determined separately in the sample and reference cuvettes. In some preparations, total heme content of microsomal suspensions was quantified by the pyridine hemochromogen method [11] using a molar absorption coefficient of 34,200 l/(mol × cm) for the A_{557nm} minus A_{575nm} difference.

Titration of sulfhydryl groups. Sulfhydryl groups were quantified by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) using a molar absorption coefficient of 13,600 l/(mol × cm) at 412 nm [12].

Statistical analyses. Differences between experimental groups were examined by Student's *t*-test; one-tailed probability limit was set at $P < 0.05$.

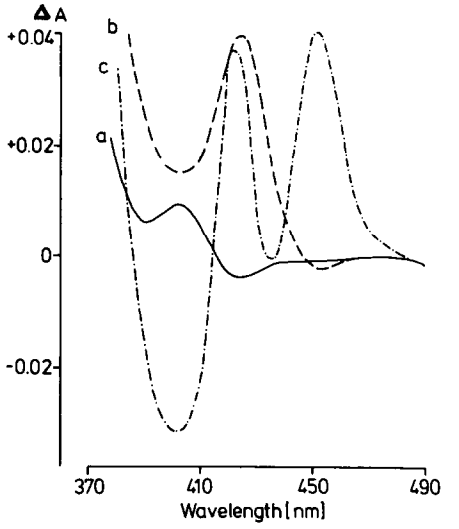

Fig. 2. Ebselen-induced spectral changes in liver microsomes. Microsomal suspensions (1 mg protein/ml) were divided between the sample and reference cuvettes. Curve (a) results after addition of DMSO to the reference and 200 μM ebselen to the sample cuvette; curve (b) results after following reduction of both the sample and reference cuvettes with dithionite; curve (c) is produced after gassing the sample cuvette with CO.

RESULTS

Ebselen-induced difference spectra

After addition of ebselen at 100 μM final concentration to rat liver microsomes in the sample cuvette, none of the typical substrate- or inhibitor-specific difference spectra (type I, reverse-type I, or type II) can be detected. Rather, a unique spectral response is induced which can be characterized by a quick transformation of the initial transient broad peak at 435 nm and the trough at 390 nm to the final conformation showing a peak at 405 nm and two troughs at 385 and 425 nm (Fig. 1). Usually, development of this difference spectrum is completed after 10 min of ebselen incubation with uninduced liver microsomes. In the presence of dithioerythritol (100 μM final concentration in both the sample and reference cuvettes), ebselen (100 μM in the sample cuvette only) is not able to develop this typical spectrum (data not shown). This may be an indication that sulfhydryl groups of the exogenously added dithioerythritol react with the ebselen molecule thus preventing ebselen interaction with the sulfhydryl groups of microsomal proteins. Indeed, addition of 100 μM ebselen reduces the amount of titratable SH groups of 50 μM dithioerythritol from 99 to 3 μM.

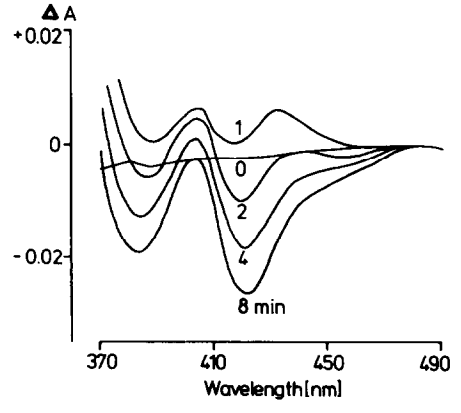

Fig. 1. Time-dependent (0–8 min) development of ebselen-induced difference spectra. Microsomal suspensions (2 mg protein/ml) were divided between the sample and reference cuvettes (0 minutes = baseline), and difference spectra were continuously recorded after addition of DMSO to the reference and 100 μM ebselen to the sample cuvette.

Ebselen-induced cytochrome P-450 *to* P-420 *conversion*

The difference spectrum obtained after reduction of ebselen-pretreated (100 μM in the sample cuvette) microsomes with sodium dithionite shows a single peak at 427 nm and a trough at 403 nm indicative of the presence of reduced cytochrome P-420 (Fig. 2, curve b). Addition of carbon monoxide to the sample cuvette results in a difference spectrum showing two peaks at 420 and 450 nm indicative of partial conversion of cytochrome P-450 into P-420 (Fig. 2, curve c). If ebselen is added after preceding reduction of microsomal suspensions or, alternatively, together with the reducing agent, this cytochrome P-450 to P-420 conversion does not occur (data not shown). Dithioerythritol can partly prevent the ebselen-induced cytochrome P-450 to P-420 conversion (see below).

The appearance of the ebselen-induced difference spectrum (Fig. 3a), the formation of reduced cytochrome P-420 (Fig. 3b), and the decrease of cyto-

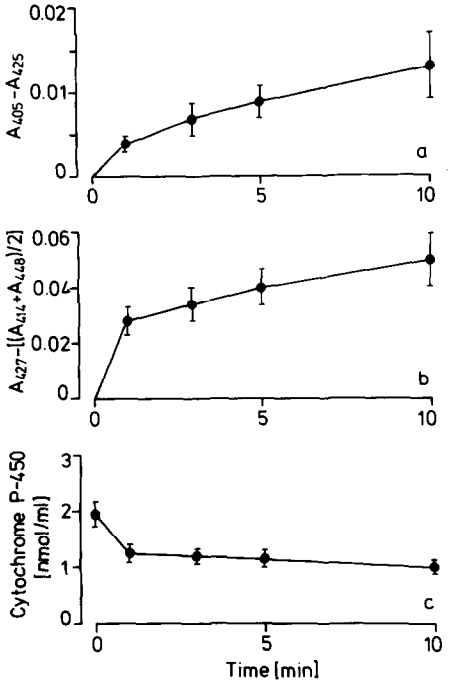

Fig. 3. Comparison of the time-dependent development of the ebselen-induced difference spectrum (a), of the ebselen-induced appearance of the reduced-cytochrome P-420 spectrum (b), and of the ebselen-induced cytochrome P-450 loss (c). Organization of the experiment was as outlined in Fig. 2. Microsomal suspensions contained 2 mg protein/ml; final ebselen concentration in the sample cuvette was 100 μM. Means ± SEM from N = 5 independent preparations.

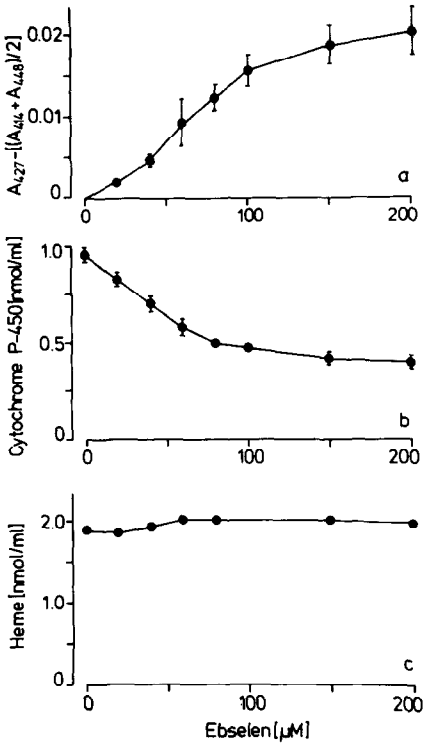

Fig. 4. Comparison of the concentration-dependent development of the ebselen-induced appearance of the reduced-cytochrome P-420 spectrum (a), of the ebselen-induced cytochrome P-450 loss (b), and of total heme concentrations (c). Microsomal suspensions contained 1 mg protein/ml. Means ± SEM from N = 4 independent experiments for (a) and (b), means from N = 2 independent experiments for (c).

chrome P-450 concentration (Fig. 3c) develop roughly parallel in time. Ebselen concentration required for half-maximal induction of the reduced-cytochrome P-420 difference spectrum is about 60 μM (Fig. 4a). Half-maximal loss of microsomal cytochrome P-450 occurs at about 50 μM ebselen concentration (Fig. 4b). Total heme content of microsomal suspensions is not affected by ebselen (Fig. 4c).

Obviously, microsomal cytochrome P-450 cannot be completely converted to P-420 even in the presence of high ebselen concentrations. There is a portion of at least 45% of the total cytochrome P-450 population that cannot be transformed by ebselen (Fig. 4b). This portion remains roughly constant independent of the initial cytochrome P-450 (or microsomal protein) and ebselen concentration ratios (Fig. 5). In phenobarbital- or methylcholanthrene-induced microsomes, however, the percentage of cytochrome P-450 to P-420 conversion amounts to 73% and 64%, respectively, compared to only 52% in control microsomes (Table 1).

Protective effect of bovine serum albumin and sulfhydryl compounds

Serum albumin can abolish the effects of ebselen on microsomal cytochrome P-450 in a concentration-dependent manner. A final concentration of 500 μM albumin is required to completely neutralize the effect of 100 μM ebselen, although 80 μM albumin is sufficient to cause a significant suppression of this action of ebselen (Table 2).

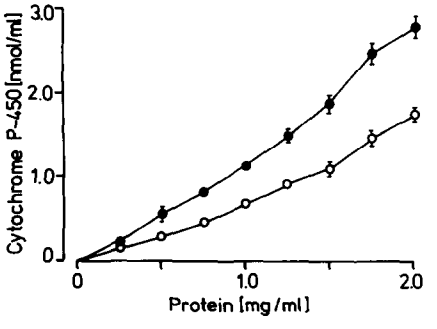

Fig. 5. Dependence of ebselen-induced cytochrome P-450 inactivation on ebselen/microsome concentration ratio. Solid circles = cytochrome P-450 concentration under control conditions, open circles = remaining cytochrome P-450 concentrations after addition of ebselen (constantly 100 μM) to the sample cuvette. Means ± SEM from N = 4 analyses.

Table 1. Effect of ebselen on cytochrome P-450 in uninduced (control), phenobarbital-induced (PB) and 3-methylcholanthrene-induced (MC) liver microsomes

Ebselen added	Cytochrome P-450 (pmol/ml) Control	PB	MC
None	960 ± 28	3109 ± 26	1302 ± 57
200 μM	463 ± 45	854 ± 76	466 ± 26

Microsomal suspensions (1.0 mg protein/ml final concentration) were divided between sample and reference cuvettes. Ebselen (or DMSO) was added to the sample cuvette, and the reference cuvette received DMSO only. Both cuvettes were reduced with dithionite, and the sample gassed with CO. Means ± SEM, N = 3 to 5 independent preparations.

A similar effect can be exerted by sulfhydryl compounds. For example, 125 μM cysteine significantly protects cytochrome P-450 from partial conversion to cytochrome P-420 in the presence of 40 μM ebselen. In the presence of 250 μM ebselen, however, 125 μM cysteine is ineffective; rather, 500 μM is required to prevent cytochrome P-450 conversion to P-420 (Fig. 6).

At 100 μM final concentrations, penicillamine, mercaptoethanol, glutathione (GSH) and dithioerythritol completely block the loss of cytochrome P-450 induced by 100 μM ebselen. Cysteine and dihydrolipoic acid show a significant, but not complete protection of cytochrome P-450 against ebselen, whereas glutathione disulfide (GSSG) is without effect (Table 3). Previous saturation of cytochrome P-450 with either type I-ligands (hexobarbital) or type II-ligands (benzylamine) can not prevent the conversion of cytochrome P-450 to P-420 induced by subsequent addition of 100 μM ebselen; similarly, addition of glycerol is not effective in protecting cytochrome P-450.

Interaction of ebselen derivatives with cytochrome P-450

Besides ebselen (compound number 1 in Table 4), its sulfur analogue (compound number 2), its

Table 2. Protective effect of bovine serum albumin (BSA) on cytochrome P-450 inactivation induced by 100 μM ebselen

BSA (μM)	Cytochrome P-450 (pmol/ml) Control	Ebselen	Difference
0	859 ± 19	544 ± 9	$P < 0.0005$
80	829 ± 13	619 ± 20	$P < 0.0005$
200	806 ± 15	731 ± 22	$P < 0.025$
500	750 ± 11	735 ± 6	NS

Microsomal suspensions (1.0 mg protein/ml final concentration) were mixed with TS buffer or BSA dissolved in TS buffer and given into the sample and reference cuvettes. DMSO or Ebselen were given to the sample and DMSO to the reference cuvette. Both suspensions were reduced with dithionite, and the sample gassed with CO. Means ± SEM, N = 4 independent preparations. NS = not significantly different.

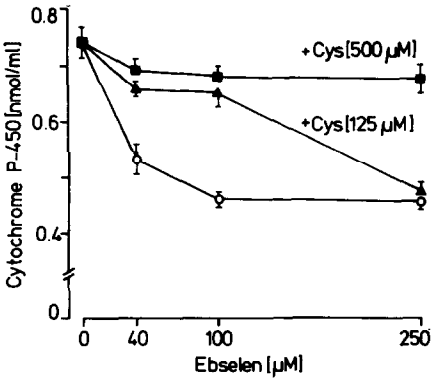

Fig. 6. Protective effect of cysteine on ebselen-induced cytochrome P-450 inactivation. Open circles = controls, solid triangles = ebselen effect in presence of 125 μM cysteine, solid squares = ebselen effect in presence of 500 μM cysteine. Microsomal suspensions (1 mg protein/ml final concentration) were mixed with TS buffer or cysteine solution and divided between sample and reference cuvettes. Ebselen was then added to the sample cuvette. Means ± SEM from N = 3 independent preparations.

halogenated analogue (compound number 3), and its *N*-methylated analogue (compound number 4) at 100 μM concentrations are all able to convert cytochrome P-450 to P-420 to a significant but varying extent (Table 4). Compounds that contain a methylated (compounds number 5 and 6) or glucuronidated (compound number 7) selenium moiety instead of the isoselenazole ring, as well as the glutathione adduct (compound number 8), obviously do not lead to a loss of liver microsomal cytochrome P-450. The same holds true for diselenides (compound number 9 in Table 4).

DISCUSSION

Difference spectra

The central heme iron of liver microsomal cytochrome P-450 is bound to the polypeptide chain of the apoprotein via a cysteinyl residue, the thiolate anion being the fifth ligand [8, 13]. This sulfur ligand–iron interaction seems to be responsible for the unique spectral characteristics of cytochrome P-450 [8]. Breaking of this ligation or denaturation of the protein result in changing of general spectral properties usually described as the cytochrome P-450 to P-420 conversion [9]. For instance, indomethacin and other non-steroidal anti-inflammatory drugs [14], captan and similar trihalogenated fungicides [15], cyclophosphamide metabolites [16], detergents and phospholipases [17, 18], heavy metals [9] and other sulfhydryl-interacting compounds [17, 18] can all cause this conversion by either interacting with the iron–thiolate ligation or with the hydrophobic environment of the cytochrome P-450. A possible physiological significance of a cytochrome P-450 to P-420 conversion has also been supposed [19].

The ebselen-induced difference spectrum (Fig. 1) points to the possibility that ebselen could interact either directly (i.e. by formation of an Se–S bond) or indirectly (i.e. by formation of an $Se–Fe^{3+}$ com-

Table 3. Protective effect of several sulfhydryl compounds (100 μM each) on cytochrome P-450 in presence of 100 μM ebselen

Compounds added	Cytochrome P-450 (pmol/ml)	Difference to controls
None	1039 ± 14 (N = 17)	
Ebselen	657 ± 17 (N = 15)	$P < 0.0005$
+ cysteine	940 ± 44 (N = 5)	$P < 0.005$
+ penicillamine	996 ± 9 (N = 3)	NS
+ mercaptoethanol	1058 ± 32 (N = 3)	NS
+ GSH	1026 ± 46 (N = 5)	NS
+ GSSG	664 ± 27 (N = 5)	$P < 0.0005$
+ dithioerythritol	980 ± 45 (N = 5)	NS
+ dihydrolipoic acid	909 ± 42 (N = 3)	$P < 0.0025$

Sulfhydryl compounds were added to microsomal suspensions (1 mg protein/ml final concentration). These suspensions were then divided between sample and reference cuvettes. DMSO or ebselen was added to the sample cuvette, and the reference cuvette received DMSO only. Both cuvettes were reduced with dithionite, and the sample gassed with CO. Means ± SEM, number of preparations in parentheses. NS = not significantly different.

plex and consecutive protonation of the S^- anion) with the thiolate group in rat liver microsomal cytochrome P-450. This spectrum which has not been previously described is phenomenologically the inverse spectrum of that observed after addition of mercaptoethanol or dithioerythritol to cytochrome P-450 [20]. The 427 nm peak appearing after reduction of ebselen-treated microsomes (Fig. 2) typically indicates the presence of reduced cytochrome P-420 [11, 18], though it can also be seen with certain reduced cytochrome P-450 metabolic intermediate complexes [21]. The final proof for ebselen-induced cytochrome P-450 to P-420 conversion comes from the CO-induced difference spectra (Fig. 2).

Modification of ebselen-induced cytochrome P-450 *to* P-420 *conversion*

In the presence of even maximal ebselen concentrations only a constant portion of the cytochrome P-450 in normal microsomes is converted to P-420 (Figs 4 and 5). The presence of sulfhydryl groups of other membrane proteins should be neutralized by high ebselen concentrations. Therefore, an explanation seems to be that only certain isoenzymes(s) of the total cytochrome P-450 population may be accessible to the ebselen molecules. This assumption is confirmed by quantitatively different responses of the total cytochrome P-450 pool to high ebselen concentrations in drug-induced microsomes (Table 1). This observation provides a further aspect to the concept of liver microsomal cytochrome P-450 heterogeneity [22].

In the presence of serum albumin, addition of ebselen is not able to elicit cytochrome P-450 to P-420 conversion (Table 2). However, it cannot be decided on the basis of these experiments whether cytochrome P-450 is protected by nonspecific binding of ebselen to albumin or whether albumin exerts its effect by a specific interaction of its sulfhydryl groups

Table 4. Effect of ebselen (compound 1) and ebselen derivatives (100 μM each) on hepatic microsomal cytochrome P-450

Compound added (number)	Cytochrome P-450 (pmol/ml)	Difference to control
None	1080 ± 64	
(1) 2-Phenyl-1,2-benzisoselenazol-3(2H)-one (ebselen)	678 ± 26	$P < 0.0025$
(2) 2-Phenyl-1,2-benzisothiazol-3(2H)-one	841 ± 42	$P < 0.025$
(3) 2-(4-Chlorophenyl)-1,2-benzisoselenazol-3(2H)-one	656 ± 30	$P < 0.0025$
(4) 2-Methyl-1,2-benzisoselenazol-3(2H)-one	859 ± 75	$P < 0.05$
(5) 2-Methylselenobenzanilide	968 ± 31	NS
(6) 4′-Hydroxy-2-methylselenobenzanilide	1031 ± 13	NS
(7) 2-Glucuronylselenobenzanilide	1035 ± 15	NS
(8) S-(2-Phenyl carbamoyl benzeneselenenyl)-glutathione	1142 ± 74	NS
(9) 2,2′-Diselenobis-(N-methyl-benzamide)	1125 ± 62	NS

Microsomal suspensions (1 mg protein/ml final concentration) were divided between sample and reference cuvettes. Compounds under investigation (dissolved in DMSO) were added to the sample cuvette, and the reference cuvette received DMSO only. Both cuvettes were reduced with dithionite, and the sample gassed with CO. Means ± SEM, N = 3 or 4 independent preparations. NS = not significantly different.

with the ebselen molecule. Nevertheless, it can be assumed that under *in-vivo* conditions, with physiological serum albumin concentrations of about 600 μM, the observed ebselen action on hepatic cytochrome P-450 is of minor relevance because the major ebselen pool may be associated with serum proteins. A similar conclusion may apply for other cytochrome P-450 transforming drugs, e.g. as studied in Ref. 14. Furthermore, ebselen may react with intracellular thiols, notably GSH, before reaching the cytochrome P-450-containing membranes of the endoplasmic reticulum *in vivo* [23]. Both mechanisms can explain the observation that dietary supply of ebselen does not affect hepatic cytochrome P-450 levels [23]. On the other hand, ebselen metabolites are detected in intact animals [24] indicating a significant entry into hepatocytes under physiological conditions.

The protective effect (Table 3, Fig. 6) of different compounds which exhibit free sulfhydryl groups supports the conclusion that ebselen can induce conversion of cytochrome P-450 to P-420 after interaction with the iron–thiolate ligation. As suggested previously, it can be assumed that the interaction with both sulfhydryl compounds and the microsomal cytochrome P-450 may be coupled to a cleavage of the Se–N bond in the ebselen molecule [7, 25, 26]. Sulfhydryl compounds have also been found to prevent cytochrome P-450 damage induced by other compounds and drugs interacting with the cytochrome P-450 thiolate group [15–18], but, to our knowledge, this is the first report describing apparent competition between hepatic cytochrome P-450 and sulfhydryl compounds with respect to binding of a seleno-organic drug. The observation that glycerol lacks any protective effect excludes the possibility that ebselen may, in addition to breaking of the iron–thiolate ligation, disturb the hydrophobic environment of cytochrome P-450 [14, 17, 18].

Specificity of interaction of ebselen derivatives with hepatic cytochrome P-450

Previous studies have shown that the primary metabolic pathway of ebselen in perfused rat liver consists of opening of the isoselenazole ring and subsequent methylation or glucuronidation of the selenium moiety [7]. These stable products of hepatic ebselen metabolism neither show significant spectral interactions with hepatic cytochrome P-450 nor favour cytochrome P-420 formation (Table 4, compounds numbers 5–9). Obviously, the selenium is not able to interact with the iron–thiolate group. In contrast, isoselenazole compounds and their sulfur analogues clearly convert part of the hepatic cytochrome P-450 population to P-420 (Table 4, compounds numbers 1–4). This pattern can be explained by the assumption that in liver microsomes, even in absence of exogenously added cofactors, compounds undergoing breakage of the Se–N or S–N bond distort the iron–thiolate ligation and cause cytochrome P-450 conversion to the inactive P-420 species.

In conclusion, partial inactivation of cytochrome P-450 by ebselen *in vitro* appears to be associated with a Se–N bond cleavage in the ebselen molecule due to interaction with SH groups of microsomal proteins *in vitro* [25]. This model provides a useful system to further study possible interactions of selenoorganic compounds with tissue sulfhydryl groups on a quantitative basis. *In vivo*, however, ebselen is obviously largely bound to plasma proteins. This may contribute to the previously reported [1] low toxicity of this substance.

Acknowledgement—We are grateful to Bettina Aufmbruch for her excellent technical assistance.

REFERENCES

1. Parnham MJ and Graf E, Seleno-organic compounds and the therapy of hydroperoxide-linked pathological conditions. *Biochem Pharmacol* **36**: 3095–3102, 1987.
2. Müller A, Cadenas E, Graf P and Sies H, A novel biologically active seleno-organic compound—I. Glutathione peroxidase-like activity *in vitro* and antioxidant capacity of PZ 51 (ebselen). *Biochem Pharmacol* **33**: 3235–3239, 1984.
3. Wendel A, Fausel M, Safayhi H, Tiegs G and Otter R, A novel biologically active seleno-organic compound—II. Activity of PZ 51 in relation to glutathione peroxidase. *Biochem Pharmacol* **33**: 3241–3245, 1984.
4. Maiorino M, Roveri A, Coassin M and Ursini F, Kinetic mechanism and substrate specificity of glutathione peroxidase activity of ebselen (PZ51). *Biochem Pharmacol* **37**: 2267–2271, 1988.
5. Müller A, Gabriel H and Sies H, A novel biologically active selenoorganic compound—IV. Protective glutathione-dependent effect of PZ51 (ebselen) against ADP-Fe induced lipid peroxidation in isolated hepatocytes. *Biochem Pharmacol* **34**: 1185–1189, 1985.
6. Cotgreave IA, Sandy MS, Berggren M, Moldeus PW and Smith MT, N-acetylcysteine and glutathione-dependent protective effect of PZ51 (ebselen) against diquat-induced cytotoxicity in isolated hepatocytes. *Biochem Pharmacol* **36**: 2899–2904, 1987.
7. Müller A, Gabriel H, Sies H, Terlinden R, Fischer H and Römer A, A novel biologically active selenoorganic compound—VII. Biotransformation of ebselen in perfused rat liver. *Biochem Pharmacol* **37**: 1103–1109, 1988.
8. White RE and Coon MJ, Oxygen activation by cytochrome P-450. *Ann Rev Biochem* **49**: 315–356, 1980.
9. De Matteis F, Loss of liver cytochrome P-450 caused by chemicals. Damage to the apoprotein and degradation of the heme moiety. In: *Heme and Heme Proteins. Handbook of Experimental Pharmacology*, Vol. 44 (Eds. De Matteis F and Aldrich WN), pp. 95–127. Springer, Berlin, 1978.
10. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
11. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein structure. *J Biol Chem* **239**: 2370–2378, 1964.
12. Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys* **82**: 70–77, 1959.
13. Hahn JE, Hodgson KO, Andersson LA and Dawson JH, Endogenous cysteine ligation in ferric and ferrous cytochrome P-450. Direct evidence from X-ray absorption spectroscopy. *J Biol Chem* **257**: 10934–10941, 1982.
14. Falzon M, Nielsch A and Burke MD, Denaturation of cytochrome P-450 by indomethacin and other nonsteroidal anti-inflammatory drugs: Evidence for a surfactant mechanism and a selective effect of a p-chlorophenyl moiety. *Biochem Pharmacol* **35**: 4019–4024, 1986.

15. Dalvi RR and Ashley WM, Protective effect of glutathione on the *in vitro* inhibition of hepatic cytochrome P-450 by captan. *Drug Chem Toxicol* **2**: 245–255, 1979.
16. Berrigan MJ, Gurtoo HL, Sharma SD, Struck RF and Marinello AJ, Protection by N-acetylcysteine of cyclophosphamide metabolism-related *in vivo* depression of mixed function oxygenase activity and *in vitro* denaturation of cytochrome P-450. *Biochem Biophys Res Commun* **93**: 797–803, 1980.
17. Ichikawa Y and Yamano T, Reconversion of detergent- and sulfhydryl reagent-produced P-420 to P-450 by polyols and glutathione. *Biochim Biophys Acta* **131**: 490–497, 1967.
18. Yu CA and Gunsalus IC, Cytochrome P-450cam. II. Interconversion with P-420. *J Biol Chem* **249**: 102–106, 1974.
19. Taniguchi H, Pyerin W and Stier A, Conversion of hepatic microsomal cytochrome P-450 to P-420 upon phosphorylation by cyclic AMP dependent protein kinase. *Biochem Pharmacol* **34**: 1835–1837, 1985.
20. Yu CA and Gunsalus IC, Cytochrome P-450cam. I. Crystallization and properties. *J Biol Chem* **249**: 94–101, 1974.
21. Elcombe CR, Bridges JW, Gray TJB, Nimmo-Smith RH and Netter KJ, Studies on the interaction of safrole with rat hepatic microsomes. *Biochem Pharmacol* **24**: 1427–1433, 1975.
22. Werringloer J and Estabrook RW, Heterogeneity of liver microsomal cytochrome P-450: The spectral characterization of reactants with reduced cytochrome P-450. *Arch Biochem Biophys* **167**: 270–286, 1975.
23. Wendel A, Otter R and Tiegs G, Inhibition by ebselen of microsomal NADPH-cytochrome P450-reductase *in vitro* but not *in vivo*. *Biochem Pharmacol* **35**: 2995–2997, 1986.
24. Fischer H, Terlinden R, Löhr JP and Römer A, Biotransformation of ebselen in rats, pigs and humans. *Xenobiotica*, in press.
25. Kamigata N, Takata M, Matsuyama H and Kobayashi M, Novel ring opening reaction of 2-aryl-1,2-benzisoselenazol-3(2H)-one with thiols. *Heterocycles* **24**: 3027–3030, 1986.
26. Fischer H and Dereu N, Mechanism of the catalytic reduction of hydroperoxides by ebselen: A selenium-77 NMR study. *Bull Soc Chim Belg* **96**: 757–768, 1987.