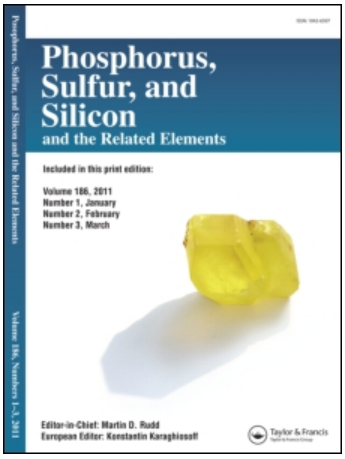

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INTERACTION OF EBSELEN WITH HEPATIC ARACHIDONATE METABOLISM IN VIVO

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INTERACTION OF EBSELEN WITH HEPATIC ARACHIDONATE METABOLISM IN VIVO

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Abstract Ebselen (PZ51, 2-phenyl-1,2-benzisoselenazol-3(2H)-one) has been previously shown to act in vitro i) as a glutathione peroxidase, ii) as an antioxidant, iii) to inhibit 5-lipoxygenase, and iiii) to isomerize leukotrienes. In this paper, the effect of ebselen and some of its analogues with different in vitro glutathione peroxidase-like activity upon mouse hepatitis models are reported. The results indicate that in vivo the predominant mechanism of action of ebselen in the liver is likely to be a specific interference with the formation of pro-inflammatory peptido-leukotrienes.

INTRODUCTION

Ebselen (PZ51) is an organic selenium compound which catalyzes the reduction of hydroperoxides with glutathione[1,2] and other thiols[3] according to the following equation:

$$2\ RSH + ROOH \rightarrow RSSR + ROH + H_2O$$

In cells, this reaction is catalyzed by the selenoenzyme glutathione peroxidase (E.C. 1.11.1.9). In microsomal suspensions ebselen was effective against iron-induced lipid peroxidation[1]. When added to isolated liver cells, ebselen protected the cells under peroxidizing conditions in the presence of glutathione or dithiothreitol, as did the antioxidant cyanidanol in the absence of thiols[4]. On the other hand, it was recognized that in vitro, the hepatic NADPH-cytochome P450 reductase was inhibited by ebselen concentrations below micromolar[5], thus interrupting the microsomal electron transfer to oxygen, i.e. lipid peroxidation.

Since the available data seemed not entirely sufficient to account for the mechanism of the anti-inflammatory *in vivo* capacity of ebselen[6], recent efforts were directed to systems more directly related to the biochemistry of the inflammatory response. A first observation was that ebselen inhibited dose dependently the formation of LTB_4 in rat neutrophil suspensions[7]. The basis of this effect was studied in more detail in subsequent studies which showed that the presence of ebselen in pharmacologically relevant concentrations catalyzed the isomerization of leukotrienes to biologically inactive *trans* isomers[8]. In accordance with these *in vitro* observed interactions of ebselen with leukotriene metabolism, the orally administered compound protected *in vivo* against a leukotriene-induced liver injury[9], i.e. galactosamine/endotoxin-induced hepatitis in mice[10].

The aim of this study was to investigate whether a general antioxidant capacity of ebselen including its GSH-Px-like activity, or an interference with the formation of pro-inflammatory mediators such as the leukotrienes might be responsible for its *in vivo* pharmacological effect.

MATERIALS AND METHODS

Male NMRI mice held on a standard diet were used in this study throughout. The severity of liver disease was assessed by measuring the enzyme activity of transaminases released into the circulation nine houres after intraperitoneal injection of 700 mg/kg galactosamine together with 50 µg/kg endotoxin. Details of the animal experiments are described in ref. 10.

Ebselen and related compounds were gifts from Dr. E. Graf, Nattermann Research Laboratories, Cologne, W. Germany. They were synthezised according to ref. 11.

RESULTS AND DISCUSSION

Mice treated with a combination of galactosamine (GalN) and endotoxin (E) developed a fulminant hepatitis as assessed by serum transaminase activity after nine hours. When the animals

were orally pretreated with ebselen, they were fully protected (Table I). The pattern of inhibition of various inhibitors of eicosanoid metabolism (Table I) suggested that liver injury was caused by a leukotriene species.

TABLE I Protective effect of orally given ebselen against galactosamine/endotoxin-induced hepatitis in mice. Influence of inhibitors of eicosanoid synthesis.

Treatment	SGPT	n
None	70 ± 30	8
Disease Control	10440 ± 8640	49
220 mg/kg Aspirin	8010 ± 8000	8
45 mg/kg Ibuprofen	7160 ± 7800	10
78 mg/kg Diethylcarbamazin	290 ± 180*	10
174 mg/kg BW 755 C	60 ± 25*	7
600 mg/kg Ebselen	140 ± 100*	12
13x10 mg/kg FPL 55712	150 ± 210*	6

Data ± S.D., n = number of animals, * $p \leq 0.001$,
Serum Glutamate/Pyruvarte Transaminase (SGPT) in U/l nine hours after GalN/E intoxication.

Hence the protective effect of ebselen might have been caused by the potency of the compound to inhibit formation of biologically active leukotrienes. The data in Table II support this interpretation. They show that in GalN pretreated mice LTD_4 substituted for endotoxin in inducing hepatitis. This leukotriene effect was blocked by the leukotriene receptor antagonist FPL 55712, but not by ebselen.

These findings indicate that ebselen is only protective before LTD_4 had been formed. The results also suggest that neither is the primary metabolic injury due to a lipid peroxidation-type pathomechanism nor does ebselen counteract it by an antioxidant action. Previous studies had shown that ebselen did not protect against peroxidatively induced hepatitis evoked by compounds such as paracetamol, CCl_4, allyl alcohol, or bromobenzene[10]. In order

to clarify the discrimination of the in vivo effect of ebselen further we tested natural as well as synthetic antioxidants in our GalN/E-model. The results are shown in Table III.

TABLE II Comparison of ebselen pretreatment and a leukotriene receptor antagonist pretreatmend in galactosamine/leukotriene D_4 induced hepatitis in mice.

Treatment	SGPT	n
None	40 ± 10	6
GalN/Endotoxin	2210 ± 2850*	16
GalN	50 ± 20	11
Endotoxin	60 ± 50	9
GalN/LTD_4	1950 ± 1740*	13
LTD_4	60 ± 10	5
GalN/LTD_4 + FPL 55712	80 ± 20	5
GalN/LTD_4 + Ebselen	1950 ± 2230*	5

* $p \leq 0.05$ compared to untreated controls;
Leukotriene dose : 50 µg/kg one hour after GalN

TABLE III Lack of efficacy of pretreatment with antioxidants or the iron depletor desferal against GalN/E-induced hepatitis in mice.

Treatment	SGPT	n
Disease control	3200 ± 2790	13
Solvent control	6430 ± 1650	6
20 mg/kg α-Tocopherolacetate[A)]	4110 ± 5720	8
200 mg/kg α-Tocopherolacetate[A)]	700 ± 840	8
20 mg/kg α-Tocopherolacetate[B)]	6820 ± 5900	6
200 mg/kg α-Tocopherolacetate[B)]	2300 ± 1260	7
Desferal[C)]	1700 ± 1730	6
Desferal without GalN/E	90 ± 50	3
Cyanidanol[D)]	3940 ± 3510	8
Butyl hydroxy toluene[A)]	2940 ± 2410	6

[A)] 2 hrs before GalN/E p.o. in oil; [B)] 3 times p.o. for three days prior to study; [C)] 6 times 500 mg/kg s.c. twice daily for three days; [D)] 2 hrs before GalN/E p.o. in 1% tylose.

None of the compounds, even at high doses or different application regimens, had any significant protective effect. In other studies these compounds were shown to be highly protective in lipid peroxidation-mediated acute hepatic injury. The findings in Table I and III clearly argue against an unspecific antioxidant pharmacological principle of the ebselen protection.

We were interested in extending our knowledge on the process by which systemic leukotriene D_4 finally leads to organ injury. Since one of the predominant properties of cysteinyl-leukotrienes is vasoconstriction we pursued the hypothesis that a transient ischemia followed by a reperfusion period might be associated with the hepatotoxic consequences of leukotriene production. According to the currently debated basic assumptions of this concept, extracellulary acting peroxide- or radical scavengers should be able to block the chain of deleterious events. The results in Table IV favor this view, although alternative explanations remain open. Allopurinol, which is capable both of inhibiting the O_2^- producing xanthine/xanthineoxidase system as well as of scavenging OH· radicals, was also effective as a protectant in our model.

TABLE IV Prevention of GalN/E hepatitis in mice by pretreatment with the xanthine oxidase inhibitor allopurinol or with reactive oxygen scavenging enzymes.

Pretreatment	SGPT	n
Untreated control	40 ± 10	10
Disease control	4970 ± 5280	19
Allopurinol[A)]	120 ± 25*	6
Superoxide dissmutase[B)]	90 ± 30*	8
Catalase[C)]	170 ± 210*	8
Bovine serum albumin[D)]	6650 ± 8840	4

[A)] 100 mg/kg i.p. 24 and 1 hr prior to GalN/E

[B)] 33000 units/kg i.v. in PBS 1 hr prior to GalN/E

[C)] 1 million units/kg in PBS 1 hr prior to GalN/E

[D)] 15 mg/kg i.v. in PBS 1 hr prior to GalN/E

* $p \leq 0.05$ compared to disease control;

PBS = phosphate buffered saline

Since ebselen did not exhibit O_2^- dismutating activity *in vitro*[3], the therapeutic principle of this compound *in vivo* seems to be narrowed down to its specific interaction with leukotriene formation. Two alternative mechanisms derived from *in-vitro* studies are available: both show similar concentration dependencies and would result in similar consequences, i.e. the inhibition of 5-lipoxygenase[7] or isomerization of leukotrienes[8].

Finally, we compared the *in-vivo* protective effect of ebselen derivatives with different GSH-peroxidase-like activities in our *in-vivo* model (Table V).

Table V Relative *in-vitro* GSH-peroxidase activity of ebselen and derivates compared to their *in-vivo* efficiacy against GalN/E hepatitis in mice.

Pretreatment[a]	GSH-Px activity (%)	SGPT[1)]
None	-	4780 ± 3620
Ebselen	= 100%	500 ± 360*
NAT 02-801[b)]	770%	420 ± 490*
NAT 02-707[c)]	360%	3320 ± 2900*

* $p \leq 0.01$, n= 8; [a)] 50 mg/kg p.o. 1 hr prior to GalN/E

[b)] 2,2-diselenobis-N,N-ethyl(4-fluorophenyl)benzamide

[c)] 2,2-diselenobis-N-dimethylbenzamide

[1)] 9 hrs after GalN/E

The available limited data seem to indicate that no direct correlation between hydroperoxide reducing activity *in vitro* and prophylactic efficacy against hepatitis *in vivo* exists. However, care must be taken with this interpretation since absorption, distribution, elimination and dose-response curves of the compounds may strongly influence the *in-vivo* results.

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