Transpl Int (1997) 10: 96–102

ORIGINAL ARTICLE

Michitaka Ozaki M.D.
Michio Nakamura M.D.
Satoshi Teraoka M.D.
Kazuo Ota M.D.

Ebselen, a novel anti-oxidant compound, protects the rat liver from ischemia-reperfusion injury

Received: 23 May 1996
Received after revision: 8 October 1996
Accepted: 28 October 1996

M. Ozaki (✉) · M. Nakamura
S. Teraoka · K. Ota
Department of Surgery III,
Tokyo Women's Medical College,
8–1, Kawada-cho, Shinjuku-ku,
Tokyo 162, Japan
Fax: + 81 3 3356 0293

Abstract The present study was designed to examine the in vivo effect of ebselen on reperfusion injury to the liver. Lipid peroxidation and glutathione (GSH) levels of the reperfused liver tissue, as well as hepatocellular damage (serum GOT, GPT, LDH, and histology) were examined. The production of thiobarbituric acid-reactive substance did not increase in the 60-min-reperfused liver tissue in the ebselen group. Ebselen completely suppressed the increase in lipid hydroperoxide production in the reperfused liver tissue. After the tissue GSH level was reduced by buthionine sulphoximine, ebselen failed to suppress the lipid peroxidation of the reperfused liver tissue. Serum levels of GOT, GPT, and LDH, histological analysis, and the tissue level of GSH clearly showed that ebselen protects the reperfused liver tissue, both structurally and functionally. We conclude that ebselen's primary effect on ischemia-reperfusion injury may be due to a GSH-peroxidase-like effect and/or the inhibitory effect of leukocyte infiltration.

Key words Reperfusion · Liver · Ebselen · Antioxidant

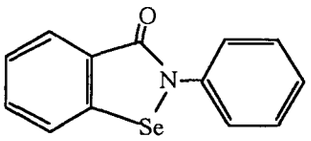
Introduction

It is well known that a tissue or organ that has been deprived of oxygen and that has been reperfused may deteriorate in its structure or function [8, 20, 30]. In clinical organ transplantation and certain surgical procedures, there may be ischemia of the organs, and these inevitably are subject to reperfusion injury. Many trials to find a method of preventing have been carried out, ischemia-reperfusion injury and various antioxidants have proven effective in preventing ischemia-reperfusion injury in different organs. Vitamins C and E are both well known anti-oxidants [5, 23]. Others, such as superoxide dismutase and catalase, have been shown to protect postischemic tissue. Some chelators have also been reported to reduce lipid peroxidative damage during reperfusion [1, 7, 22].

Reactive oxygen compounds generated in the reperfused tissue initiate the peroxidation of membrane lipids and produce lipid hydroperoxides and aldehydic substances that are both physiologically and toxicologically active [3]. The lipid peroxidation of membranes also causes the release of membrane-bound arachidonic acid, which may be rapidly metabolized to prostaglandins and leukotrienes. These lipid metabolites play important roles in the chemotaxis of leukocytes and control of vascular endothelial cell function [24].

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is a water-insoluble compound that contains selenium (Fig. 1) and that has various pharmacological effects involving antioxidant activity [10, 17]. In in vitro experiments using liver microsomes or hepatocytes, ebselen was shown to markedly reduce lipid peroxidative reactions [19, 29]. It has been shown to exhibit unique glutathione (GSH)-peroxidase-like activity [18], reducing hydrogen peroxide and other hydroperoxides with thiol cosubstrates such as GSH and N-acetylcysteine [2]. The GSH-peroxidase-like activity of ebselen has been shown to catalyze the conversion of LTB4 to an inactive isomer. It may also catalyze the reduction of endoperoxide prod-

Fig. 1 Structure of ebselen (2-phenyl-1,2-benzisoselenazol-3-(2H)-one). This is a water-insoluble compound that contains selenium within the molecule.



$C_{13}H_9NOSe$
M.W. : 274.18

ucts of lipoxygenases and the reduction of PGG_2 to PGH_2 [15]. Orally administered ebselen has been reported to inhibit the LTB_4-induced aggregation of leukocytes and ADP-induced aggregation of platelets in whole blood in the rat [25]. In rat Kupffer cells, ebselen inhibits the release of superoxide anion and nitric oxide, and it protects the graft from reperfusion injury [28]. Recently, ebselen has been reported to prevent reperfusion injury to the brain, heart, and stomach [11, 12, 27]. Though ebselen's strong effect on reperfused tissue is considered to be due to a GSH-peroxidase-like effect, the precise mechanism in vivo is still unclear. This unique compound is expected to protect the organ from lipid peroxidative injury after ischemia-reperfusion. Nevertheless, the in vivo effects of ebselen on ischemia-reperfusion injury to the liver have not yet been reported.

The present study was designed to examine the in vivo effects of ebselen on lipid peroxidative injury to the rat liver during reperfusion. Lipid peroxidation was estimated by measuring conjugated diene (fluorescent products), lipid hydroperoxides (LPO), and thiobarbituric acid-reactive substance (TBA-RS) of the hepatic tissue. Tissue GSH level (reduced and oxidized form) and hepatocellular damage (serum GOT, GPT, and LDH levels, and histology) were also examined.

Materials and methods

Ebselen was supplied by Daiichi Pharmaceutical (Tokyo, Japan). A reduced form of glutathione (GSH), buthionine sulphoximine (BSO), glyoxalase 1, methylglyoxal, reduced form of nicotineamide adenine dinucleotide phosphate (NADPH), glutathione reductase and thiobarbituric acid (TBA) were purchased from Sigma (St. Louis, Mo., USA). The hemoglobin-methylene blue (HMB) test kit for the LPO assay was obtained from Kyowa Medex (Tokyo). All other chemicals were of analytical grade and were used without further purification.

Experimental procedures

Male Wistar rats, weighing approximately 250 g, were fasted overnight. Ebselen was dissolved in liquid paraffin and administered orally, 40–200 mg/kg body weight (BW), in a volume of 1 ml. In a control group, 1 ml of liquid paraffin was administered orally. BSO or GSH was administered intraperitoneally 2 h or 1 h before ischemia (2 mmol/kg BW) respectively. Anesthesia was induced with an intraperitoneal injection of nembutal (pentobarbital sodium, 60 mg/100 g BW). After laparotomy, all vessels (hepatic artery, portal vein, and bile duct) to the left and median liver lobes were clamped, according to the method of Hasselgren et al. [9]. After 60 min of hepatic ischemia, these vessels were unclamped and circulation was restored. Liver specimens for the biochemical analysis were excised before and after ischemia, and after reperfusion. Blood samples were taken within 1 min of hepatic reperfusion and again after 60 min of reperfusion; serum levels of GOT, GPT, and LDH were also measured at this time. In each group, experiments were performed five times with groups of five rats. Liver specimens for histological analysis were excised after 20 min, 120 min, and 9 h of reperfusion.

The experimental protocol was approved by the Animal Studies Committee of Tokyo Women's Medical College.

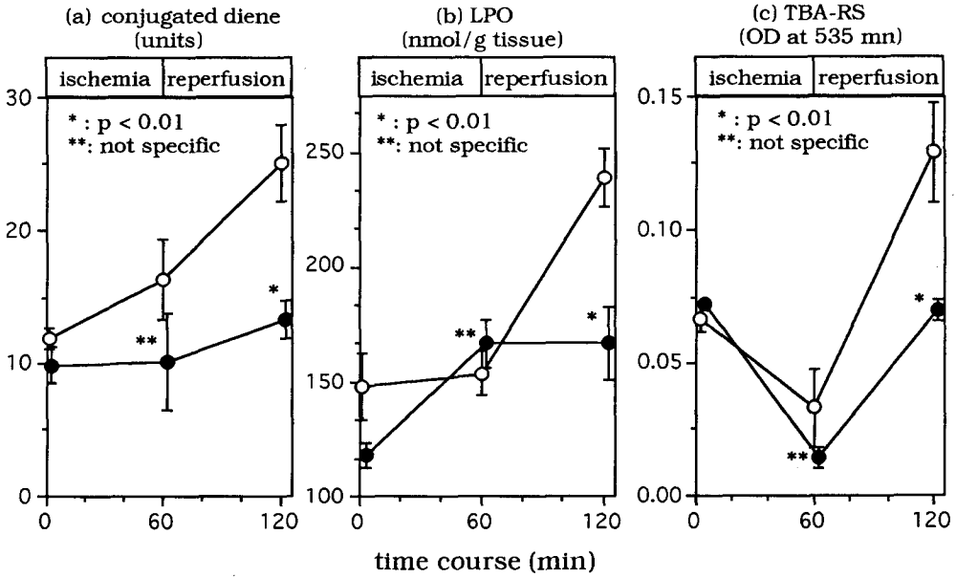


Fig. 2a–c Lipid peroxidation of the reoxygenated liver tissue. The production of: **a** diene conjugates, **b** LPO, and **c** TBA-RS in the liver tissue during ischemia and reperfusion is shown. Ebselen, 100 mg/kg BW, was administered orally 60 min before hepatic ischemia. After 60 min of ischemia, the liver was reoxygenated for 60 min. All data shown are expressed as means ± SE for five specimens in each group (○ control group, ● ebselen group)

Table 1 Glutathione content of the liver tissue during ischemia and reperfusion. Date are expressed as means ± SE for five specimens in each group (GSH reduced form of glutathione, GSSG oxydised form of glutathione, BSO buthionine sulphoximine)

	GSH GSSG (μmol/g tissue)		
	Ischemia 0 min	Ischemia 60 min	Reperfusion 60 min
Control	11.158 ± 0.888 0.378 ± 0.111	5.830 ± 1.432 0.272 ± 0.220	2.775 ± 1.235 0.071 ± 0.040
Ebselen	12.239 ± 1.995 0.194 ± 0.080	8.243 ± 0.989 0.145 ± 0.050	5.245 ± 1.253 0.157 ± 0.047
Ebselen + BSO	2.405 ± 1.667[a] 0.035 ± 0.022	1.440 ± 0.038 0.023 ± 0.009	1.526 ± 0.971 0.070 ± 0.011
Ebselen + GSH	14.677 ± 1.897 0.230 ± 0.108	5.668 ± 2.086 0.115 ± 0.076	6.326 ± 1.272 0.186 ± 0.086

[a] Significant at 95 % confidence level (dunnett's test)

Assay of diene conjugates, LPO, and TBA-RS of the liver tissue

Conjugated diene (fluorescent peroxidative products) were measured with a modified version of the method used by Fletcher et al. [4]. Liver tissue was blotted to remove excess moisture, and samples of approximately 0.2 g were weighed. Chloroform and methanol, 2:1 (v/v), were added in a volume-to-weight ratio of 20:1. Tissue was homogenized with a Teflon homogenizer for 1 min. An equal volume of water was then added. The extract was mixed thoroughly on a vortex mixer at high speed and transferred to a centrifuge tube, where it was centrifuged at 3000 rpm for 1–2 min. A 1-ml aliquot of the chloroform-rich layer was mixed with 0.1 ml of methanol in a quartz cuvette, and the fluorescence spectra were recorded after standardization with quinine sulphate at a concentration of 1 μg/ml in 0.05 M-H_2SO_4 and at an excitation wavelength of 380 nm and an emission wavelength of 460 nm. Liver specimens were homogenized with ten volumes of 1.15 % KCl solution and then assayed for TBA-RS and LPO. We assayed the TBA-RS using the method of Ohkawa et al. [21] and we assayed the tissue LPO with the HMB test kit (Kyowa Medex, Tokyo). The HMB test has a high specificity for peroxides because it uses the peroxidase activity of hemoglobin. When hemoglobin reduces peroxides to the corresponding alcohols, N-methylcarbamoyl derivatives of methylene blue (leuco form) are oxidized and colored blue [13].

Assay of the liver tissue

The liver specimen was homogenized with five volumes of 6 % perchloric acid and neutralized with phosphate solution. Glutathione was assayed with the method of Klotsh and Bergmeyer [14].

Histological analysis

The liver specimen was fixed with 10 % buffered formalin solution, then embedded in paraffin and stained with H & E.

Statistical analysis

Analysis of variance and Student's *t*-test (two-sided) for unpaired values were used for testing statistical significance. A P value below 0.05 was considered significant. As for the analyses of the differences between multiple groups, Dunnett's test or Scheffe's test were used and considered significant at a 95 % confidence level.

Results

Lipid peroxidation of the liver tissue during ischemia and reperfusion

In the control group, the production of conjugated diene of the liver tissue was increased during hepatic reperfusion, whereas it was suppressed in the ebselen group (control 25.1 ± 2.8 units, ebselen 13.3 ± 1.3 units, $P < 0.01$ vs control). LPO production was also significantly increased in the liver tissue after reperfusion in the control group. Yet, the administration of ebselen almost completely suppressed the increase in LPO production in the reperfused liver tissue (control 238.7 ± 12.5 nmol/g tissue, ebselen 167.3 ± 16.1 nmol/g tissue, $P < 0.01$ vs control). In the control group, TBA-RS production in the liver tissue was significantly increased by hepatic reperfusion. The production of TBA-RS was not increased after 60 min of reperfusion in the ebselen group (control 0.129 ± 0.019 OD, ebselen 0.070 ± 0.004 OD at 535 nm, $P < 0.01$ vs control; Fig. 2).

Dose-dependent effects of ebselen on lipid peroxidation of the reperfused liver tissue

Previous administration of ebselen at 40 mg/kg BW failed to suppress the production of TBA-RS in reperfused liver tissue (0.131 ± 0.015 OD at 535 nm). However, administration of more than 100 mg/kg BW did suppress it effectively (ebselen 100 mg/kg: 0.070 ± 0.004 OD, 200 mg/kg: 0.067 ± 0.005 OD at 535 nm). Similar results were obtained for the LPO assay (data not shown).

Table 2 Serum GOT, GPT, and LDH levels (IU/l) after hepatic ischemia. Data are expressed as means ± SE for five specimens in each group

* $P < 0.01$ vs control

	After ischemia			After reperfusion (120 min)		
	GOT	GPT	LDH	GOT	GPT	LDH
Control	497 ± 35	540 ± 35	5407 ± 328	7631 ± 1686	6019 ± 1132	64787 ± 12669
Ebselen	824 ± 276	902 ± 297	10818 ± 2450	751 ± 185*	879 ± 283*	9551 ± 2718*

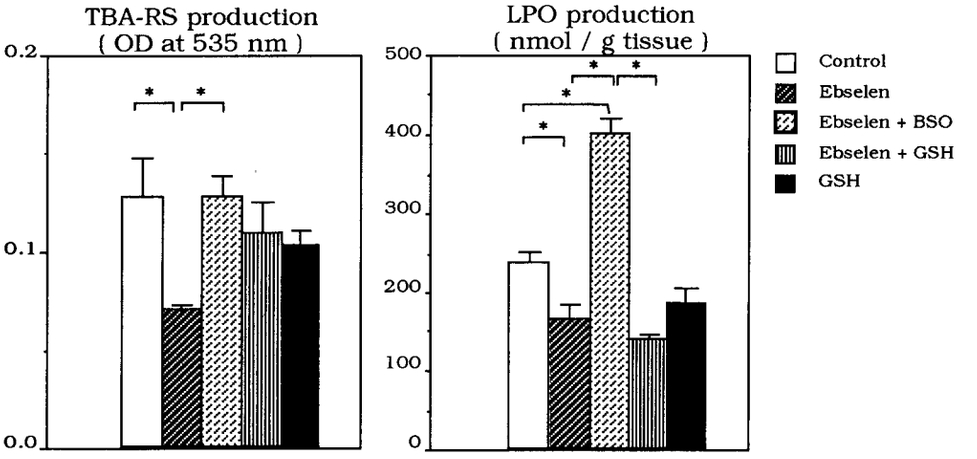


Fig. 3 Effects of BSO or GSH on Ebselen's ability to suppress lipid peroxidation. The production of TBA-RS and LPO in postischemic liver tissue is shown. Ebselen, 100 mg/kg BW, was administered orally 60 min before hepatic ischemia. BSO or GSH was administered intraperitoneally 120 min before hepatic ischemia. All data shown are expressed as means ± SE for five specimens in each group. * Significant at 95 % confidence (Scheffe's test)

Effects of BSO or GSH on ebselen's ability to suppress lipid peroxidation

Ebselen failed to suppress the production of TBA-RS and LPO in the reperfused liver tissue when either BSO or GSH was also administered. When administered alone, GSH showed a mild suppressive effect on LPO and TBA-RS production (Fig. 3).

Gluthathione level in the liver tissue during ischemia and reperfusion

In the control group, the tissue GSH level was reduced during hepatic ischemia and continued to drop during reperfusion. The administration of BSO induced a decrease in the tissue GSH level. During ischemia, tissue GSH levels were reduced in all groups. In the reperfused liver tissue, both tissue GSH and oxydised form of glutathione (GSSG) levels remained comparatively high in the ebselen group and in the ebselen + GSH group compared with those in the control group (Table 1).

Serum levels of GOT, GPT, and LDH after hepatic ischemia

Just after hepatic reperfusion, serum levels of GOT, GPT, and LDH were moderately high in both the control and ebselen groups. Two hours after hepatic reperfusion, serum levels of GOT, GPT, and LDH were markedly elevated in the control group but not in the ebselen group (Table 2).

Histological analysis of the reperfused liver tissue

Histological analysis of the 20-min-reperfused liver tissue showed no apparent difference between the control and ebselen groups. In both groups, mild edema was observed with little leukocyte infiltration. Histological analysis of the 120-min-reperfused liver tissue revealed that ebselen protected the reperfused liver tissue from the reperfusion injury. In the control group, leukocyte infiltration in the portal areas, focal hepatocellular necrosis, and extended edema were observed (Fig. 4 a). However, little leukocyte infiltration in the portal areas, mild edema in central areas, and no apparent necrosis were observed in the ebselen group (Fig. 4 b). After 9 h of reperfusion, the extended necrotic areas and leukocyte infiltration in the liver tissue observed in the control group were much less observed in the ebselen group.

Discussion

The present study reveals that a seleno-organic compound, ebselen, exhibits strong protective effects against reperfusion injury to the liver.

Ebselen strongly suppressed an increase in the production of conjugated diene, LPO, and TBA-RS in the reperfused liver tissue. Ebselen was especially successful in the complete suppression of LPO production during reperfusion. BSO is well known to be an effective inhibitor of GSH synthesis [16], and it successfully reduced the GSH levels of the pre- and postischemic liver tissue. With the addition of BSO, the suppressive effect of ebselen on lipid peroxidation of the reperfused liver tissue almost disappeared. In particular, compared with the control group, LPO production in the reperfused liver tissue in the ebselen + BSO group increased significantly. With the addition of GSH, the tissue level of

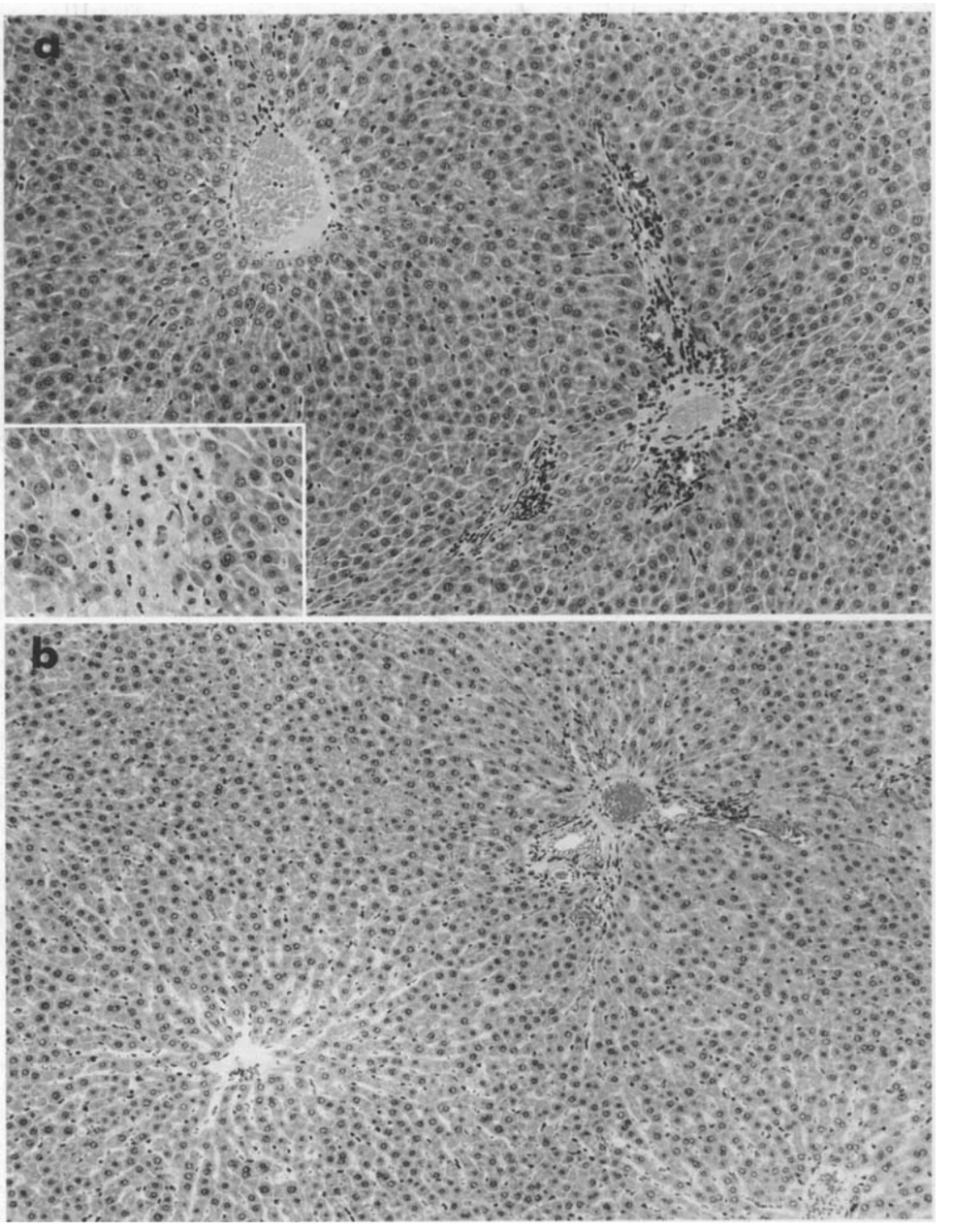


Fig. 4 a,b Photomicrographs of the postischemic liver tissue at 120 min of reperfusion: **a** control; extended edema, focal necrosis, and leukocyte infiltration of the postischemic liver tissue are observed (H&E X 40). *Inset* shows a high-power photomicrograph of focal hepatocellular necrosis of the postischemic liver tissue (X 100); **b** Ebselen, administered orally (100 mg/kg BW) 60 min before hepatic ischemia. Moderate edema and little leukocyte infiltration are observed (H&E × 40)

GSH increased, and the peroxidative tissue was slightly protected by GSH alone. However, ebselen administered together with GSH failed to further suppress lipid peroxidation. These findings suggest that ebselen reacts directly with lipid hydroperoxides in the presence of GSH. It also suggests that GSH either works as an antioxidant by itself or is involved in other antioxidant mechanisms. In the ebselen and ebselen + GSH groups, high GSSG levels were observed in the reperfused liver tissue. One possible explanation is that ebselen reacts with hydroperoxides to change to the reduced form, coupled with the reaction of GSH to GSSG. This would support the idea that ebselen exhibits GSH-peroxidase-like activity, not only in vitro but also in vivo.

There is, however, a report that describes ebselen's ability to scavenge free radicals and singlet oxygen [26]. In the present study, the production of conjugated diene was also rather suppressed compared with that of TBA-RS. This would suggest that ebselen also has the in vivo ability to scavenge reactive oxygen compounds and, as

a result, that it suppresses the initiation of lipid peroxidation of the tissue. However, the in vivo scavenging capacity of ebselen remains to be studied.

Serum levels of GOT, GPT, and LDH just after hepatic ischemia and after 120 min of ischemia showed that ebselen successfully suppressed the peroxidative hepatocellular injury that occurs during reperfusion.

Histological analysis also showed that ebselen protects the liver from ischemia-reperfusion injury. Both the infiltration of leukocytes to the reperfused tissue and necrosis seemed to be reduced by administering ebselen, though no quantitative analysis was performed. This may be explained by ebselen's anti-oxidant effect and inhibitory effect against leukocyte infiltration.

Ebselen's ability to suppress inflammation and/or lipid peroxidation may be due to a GSH-peroxidase-like effect, its ability to scavenge reactive oxygen compounds, and/or its ability to inhibit leukocyte infiltration. The former appears to be its most important effect and the one that the present study supports as an explanation for ebselen's suppression of hepatic ischemia-reperfusion injury.

The reduction in tissue leukocyte infiltration and focal necrosis by ebselen may be explained by its inhibitory effect on leukocyte aggregation, as well as by its antioxidant effect on lipid peroxidation in the early stage of reperfusion. Ebselen may protect reperfused liver tissue by suppressing leukocyte aggregation, preserving peripheral circulation. Tissue leukocyte infiltration during reperfusion seems to occur by the expression of adhesion molecules onto the surface of endothelium following lipid peroxidative cellular injury [6]. By suppressing both primary lipid peroxidative cellular injury and the resulting expression of adhesion molecules, ebselen may be able to inhibit leukocyte infiltration. However, precise mechanisms are still unclear.

The present study shows that ebselen successfully suppresses both lipid peroxidative damage, mainly by its GSH-peroxidase-like effect, and leukocyte infiltration into the reperfused liver tissue. Because this compound has been reported to have similar effects on other organs (brain, heart, stomach) [11, 12, 27], it has been suggested that ebselen does not target the liver parenchyma but mainly the vascular system of the liver.

In conclusion, ebselen exhibits a strong antioxidant effect on hepatic ischemia-reperfusion injury. Though further experiments must be done, ebselen appears to be an effective antioxidant that prevents oxidative tissue damage encountered in the course of various surgical treatments, especially organ transplantation. The present study supports the theory that ebselen's effect on ischemia-reperfusion injury to the liver is mainly due to its GSH-peroxidase-like effect, together with its ability to inhibit leukocyte infiltration. What remains unclear is the role that ebselen's ability to scavenge reactive oxygen compounds plays in protecting postischemic tissue.

References

1. Burk RF, Nishiki K, Lawrence RA, Chance B (1978) Peroxide removal by selenium-dependent and selenium-independent glutathione peroxidases in hemoglobin-free perfused rat liver. J Biol Chem 253: 43–46
2. Cotgreave IA, Sandy MS, Berggres M, Modeus P, Smith MT (1987) N-acetylcysteine and glutathione-dependent protective effects of PZ-51 (Ebselen) against diquat-induced cytotoxicity in isolated hepatocytes. Biochem Pharmacol 36: 2899–2904
3. Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and Biochemistry of 4-hydroxynonenal, malonealdehyde and related aldehydes Free Radic Biol Med 11: 81–128
4. Fletcher BL, Dillard CJ, Tappel AL (1973) Measurement of fluorescent lipid peroxidation products in biological systems and tissues. Anal Biochem 52: 1–9
5. Fukuzawa K, Takase S, Tsukatani H (1985) The effect of concentration on the antioxidant effectiveness of alpha-tocopherol in lipid peroxidation induced by superoxide free radicals. Arch Biochem Biophys 240: 117–120
6. Grace PA (1994) Ischemia-reperfusion injury. Br J Surg 81: 637–647
7. Granger GN, Hollwarth ME (1986) Ischaemia-reperfusion injury: role of oxygen derived free radicals. Acta Physiol Scand Suppl 548: 47–63
8. Granger DN, Rutili G, McCord JM (1981) Superoxide radicals in feline intestinal ischemia. Gastroenterology 81: 22–29
9. Hasselgren PO, Hellman A, Jennische E, Nordstrom G (1984) Failure of an increased dose of ATP-$MgCl_2$ to improve protein synthesis and transmembrane potential in the postischemic liver. J Surg Res 37: 409–414
10. Hayashi M, Slater TF (1986) Inhibitory effects of ebselen on lipid peroxidation in rat liver microsomes. Free Radic Res Commun 2: 179–185
11. Hoshida S, Kuzuya T, Nishida M, Yamashita N, Hori M, Kamada T, Tada M (1994) Ebselen protects against ischemia-reperfusion injury in a canine model of myocardial infarction. Am J Physiol 267: H2342–H2347
12. Johshita H, Sasaki T, Matsui T, Hanamura T, Masayasu H, Asano T, Takakura K (1990) Effects of ebselen (PZ51) on ischaemic brain oedema after focal ischaemia in cats. Acta Neurochir Suppl (Wien) 51: 239–241
13. Kanazawa K, Inoue N, Ashida H, Mizuno M, Natake M (1989) What do thiobarbituric acid and hemoglobin-methylene blue test evaluate in the endogenous lipid peroxidation of rat liver? J Clin Biochem Nutr 7: 69–79
14. Klotsh H, Bergmeyer HU (1974) Methods of enzymatic analysis, vol. 3. Academic Press, New York
15. Kuhl P, Borbe HO, Fischer H, Romer A, Safayhi H (1986) Ebselen reduces the formation of LTB4 in human and porcine leukocytes by isomerisation of its SS-12R-6-trans isomer. Prostaglandins 31: 1029–1040

16. Meister A (1988) Glutathione metabolism and its selective modification. J Biol Chem 263: 17205–17208
17. Muller A, Cadenas E, Graf P, Sies H (1984) A novel biologically active seleno-organic compound- I. Biochem Pharmacol 33: 3235–3239
18. Muller A, Gabriel H, Sies H (1985) A novel biologically active selenoorganic compound – IV. Biochem Pharmacol 34: 1185–1189
19. Narayanaswami V, Sies H (1990) Antioxidant activity of Ebselen and related selenoorganic compounds in microsomal lipid peroxidation. Free Radic Res Commun 10: 237–244
20. Nordstrom G, Seeman T, Hasselgren PO (1985) Beneficial effect of allopurinol in liver ischemia. Surgery 97: 679–684
21. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358
22. Ozaki M, Fuchinoue S, Teraoka S, Ota K (1994) Mobilization of low molecular weight iron and peroxidative damage during ischemia and reoxygenation of rat liver. Transplant Proc 26: 918–921
23. Ozaki M, Fuchinoue S, Teraoka S, Ota K (1995) The in vivo cytoprotection of ascorbic acid against ischemia/reoxygenation injury of rat liver. Arch Biochem Biophys 318: 439–445
24. Parnham MJ, Graf E (1987) Seleno-organic compounds and the therapy of hydroperoxide-linked pathophysiological conditions. Biochem Pharmacol 36: 3095–3102
25. Sakuma K, Masayasu H, Shibata H, Shida S (1989) Ebselen, a seleno-organic compound, inhibits leukocyte aggregation and reduces the plasma leukotriene B4 level in humans and rats. In: Wandel A (ed) Selenium in biology and medicine. Springer-Verlag, Berlin, pp 153–156
26. Sies H (1993) Ebselen, a selenoorganic compound as glutathione peroxidase mimic. Free Radic Biol Med 14: 313–323
27. Ueda S, Yoshikawa T, Takahashi S, Naito Y, Oyamada H, Takemura T, Morita Y, Tanigawa T, Sugino S, Kondo M (1990) Protection by seleno-organic compound, Ebselen, against acute gastric mucosal injury induced by ischemia-reperfusion in rats. Adv Exp Med Biol 264: 187–190
28. Wang JF, Komarov P, Sies H, Groot H (1992) Inhibition of superoxide and nitric oxide release and protection from reoxygenation injuy by Ebselen in rat Kupffer cells. Hepatology 15: 1112–1116
29. Wendel A, Fausel M, Safayhi H, Tiegs G, Otter R (1984) A novel biologically active seleno-organic compound-II. Biochem Pharmacol 33: 3241–3245
30. Younes M, Strubelt O (1988) The involvement of reactive oxygen species in hypoxic injury to rat liver. Res Commun Chem Pathol Pharmacol 59: 369–372