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Susceptibility of Mice to Bacterial and Fungal Infections after Intragastric Administration of Ebselen

R. NOZAWA, M. ARAI, R. KURUTO, T. MOTOHASHI* AND H. MASAYASU*

*Laboratory of Microbiology, University of Shizuoka School of Food and Nutritional Sciences, Yata, Shizuoka-shi, and *Daiichi Pharmaceutical Co. Ltd, Kitakasai, Edogawa-ku, Tokyo, Japan*

Abstract

The seleno-organic compound ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) has anti-inflammatory activity and exhibits glutathione peroxidase-like activity in-vitro. Ebselen inhibited candidacidal activity over the same range of concentrations as it inhibited the production of microbicidal H_2O_2 by human neutrophils and macrophage-like cells. Therefore, the long-term administration of ebselen might be expected to induce an immunocompromised state in the host. To examine such a possibility, mice (5-weeks-old ddY, male) were given daily intragastric doses of 0, 10 or 100 mg kg^{-1} ebselen for 21 days and then infected intraperitoneally with *Candida albicans* (10^8 cells/mouse), *Pseudomonas aeruginosa* (1.5×10^7 cells/mouse) or methicillin-resistant *Staphylococcus aureus* (5×10^8 cells/mouse). Ebselen at none of the tested doses affected the increase in body weight of mice during administration of the drug. No evidence was obtained that mice became more susceptible to the various microorganisms after the administration of ebselen at any tested dose.

Selenium is an essential trace element for mammals. It is present in glutathione (GSH) peroxidase, which catalyses the reduction of a variety of hydroperoxides. The seleno-organic compound 2-phenyl-1,2-benzisoselenazol-3(2H)-one (ebselen) exhibits GSH peroxidase-like activity in-vitro (Müller et al 1984; Parnham & Kindt 1984; Wendel et al 1984) and it has anti-inflammatory activity in various animal models (Schalkwijk et al 1986; Cotgreave et al 1988; Akasaki et al 1989; Kurebayashi et al 1989; Matsui et al 1990; Niederau et al 1991; Hoshida et al 1994). Reactive oxygen metabolites and peroxygenated lipid metabolites produced by inflammatory cells are deeply involved in both acute and chronic inflammatory processes (Weiss & Lobuglio 1982; Henson & Johnston 1987; Parnham & Graf 1987). Ebselen inhibits the production of these metabolites in macrophages and neutrophils (Ichikawa et al 1987; Cotgreave et al 1989; Leurs et al 1989), which are key cells in the inflammatory process. Reactive oxygen metabolites, by contrast, are essential for oxidative killing of micro-organisms by these phagocytic cells. We studied the effects of ebselen on the candidacidal activity of human peripheral blood neutrophils and macrophage-like cells, and we found that a high concentration (more than 30 μM) of ebselen inhibited the candidacidal activity. Therefore, the long-term administration of ebselen might be expected to induce an immunocompromised state in the host. In this study, mice were treated intragastrically with various doses of ebselen for three weeks, and then they were infected with semilethal numbers of *Candida albicans*, *Pseudomonas aeruginosa* or *Staphylococcus aureus* and their susceptibility to infection by these microorganisms was examined.

Correspondence: R. Nozawa, Laboratory of Microbiology and Host Defenses, University of Shizuoka School of Food and Nutritional Sciences, 52-1 Yata, Shizuoka-shi 422, Japan.

Materials and Methods

Animals

Specific pathogen-free ddY mice (male, 5-weeks-old) were obtained from SLC Japan K. K. (Hamamatsu, Japan).

Assay of candidacidal activity

Candidacidal activity was assayed as described previously (Nozawa et al 1980). In brief, phagocytic cells were seeded at 10^5 cells per well in 96-well tissue culture plates with indicated concentrations of ebselen and infected with a serially-diluted suspension of *Candida parapsilosis* JCM 1785. During the subsequent incubation at 37°C, surviving *Candida* cells overgrew in each well. The maximum number of microorganisms killed (MNMK) was determined after a 48-h incubation period.

Assay of the production of H_2O_2

Human leukaemia HL-60 cells that had been treated with 10^{-7} M 1,25-dihydroxyvitamin D_3 (VD_3) for three days (Nozawa et al 1988) were incubated with indicated concentrations of ebselen at 37°C for 30 min and then treated with 100 ng mL^{-1} phorbol myristate acetate (PMA). Released H_2O_2 was assayed by measuring the reduction in fluorescence of scopoletin in the presence of horseradish peroxidase (Nathan & Root 1977).

Administration of ebselen

Ebselen was suspended in distilled water at 0, 2·5 and 25 mg mL^{-1}, and administered intragastrically by cannulation to mice at doses of 0, 10 and 100 mg kg^{-1}. Mice were given the drug daily for 21 days and infected with microorganisms on the 21st day.

Microorganisms and infection

Candida albicans JCM 1542, *Pseudomonas aeruginosa*

Table 1. Effects of ebselen on the candidacidal activity of phagocytes.

Cells	Ebselen (μM)	MNMK* Experiment 1	Experiment 2
HL-60	0	< 16	
HL-60 + VD_3†	0	1024	640
	1	512	–
	10	1024	320
	30	–	< 10
Neutrophils	0	2048	5120
	1	> 4096	
	10	4096	5120
	30	–	320

Experiments were performed in duplicate, and mean results are shown. *Maximum number of microorganisms killed. †Treated with 10^{-7} M VD_3 for three days.

Table 2. Effects of ebselen on the PMA-induced production of H_2O_2 in HL-60 cells.

Ebselen (μM)	Production of H_2O_2 (%)
10	88
20	78
50	15
100	8

The results are the average from two separate experiments. HL-60 cells were induced to differentiate by a three-day treatment with VD_3. Amount of H_2O_2 produced by cells in the absence of ebselen was taken as 100%.

PAO-1, and methicillin-resistant *Staphylococcus aureus* (MRSA) #10 (Nozawa et al 1989) were used for infection of mice. Freshly cultivated *C. albicans*, *P. aeruginosa* and *S. aureus* cells on Sabouraud dextrose agar, nutrient agar and heart-infusion agar, respectively, were scraped off the agar, suspended in saline G (Kao & Puck 1974) and injected intraperitoneally at 10^8, $1{\cdot}5 \times 10^7$ and 5×10^8 cells/0·5 mL/mouse, respectively. Survival of mice was recorded 72 h after the injection of *C. albicans*, and 48 h after injection of *P. aeruginosa* and *S. aureus*.

Materials

VD_3 was supplied from Chugai Pharmaceutical Co. (Tokyo, Japan). PMA and horseradish peroxidase were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Results

Candidacidal activity of ebselen-treated neutrophils and macrophage-like cells

The effects of ebselen on the microbicidal activity of human neutrophils were examined with *C. parapsilosis* as the target microorganism (Table 1). Ebselen at up to 10 μM had little effect on the candidacidal activity of neutrophils, whereas ebselen at 30 μM greatly impaired this activity. Human leukemia HL-60 cells were converted to macrophage-like cells by treatment with 10^{-7} M VD_3 for three days (Nozawa et al 1988). Untreated HL-60 cells were barely able to kill *Candida* cells, whereas the cells that had been treated with VD_3 exhibited strong candidacidal activity (Table 1). Ebselen at 30 μM inhibited the candidacidal activity of the macrophage-like cells.

Inhibition by ebselen of the production of H_2O_2 by VD_3-treated HL-60 cells

The effects of ebselen on the production of H_2O_2 by VD_3-treated HL-60 cells were then studied. Ebselen at more than 20 μM greatly inhibited the production of H_2O_2 by macrophage-like cells (Table 2). The inhibitory concentration of ebselen in the case of macrophage-like cells was almost the same as that in the case of human neutrophils (Cotgreave et al 1989).

Infection with C. albicans

Mice were divided to three groups (20 or more mice per group) and given daily intragastric doses of 0, 10 or 100 mg kg^{-1} ebselen for 21 days. Increases in the body weight during the 21-day period did not differ between the groups (Table 3, experiment 1). In a preliminary experiment, 1 of 4, 4 of 4, and 3 of 4 untreated mice died 72 h after intraperitoneal injection of 10^8, $3{\cdot}3 \times 10^8$ and 10^9 *C. albicans* cells per mouse, respectively. To test the susceptibility of ebselen-treated mice to *C. albicans*, the mice were injected intraperitoneally with a semilethal number of *Candida* cells (10^8 cells) on day 21. Twelve of 20 control mice survived after 72 h. The rate of survival did not differ among the three groups (Table 4, experiment 1).

Table 3. Increases in body weight during the administration of ebselen to mice.

Experiment	Ebselen (mg kg^{-1})	Body weight (g)		
		Day 0	Day 9	Day 21
1	0	31·0 ± 1·1	34·6 ± 1·5	37·0 ± 2·1 (n = 20)
	10	30·6 ± 1·4	34·5 ± 2·0	37·1 ± 2·4 (n = 21)
	100	30·7 ± 1·4	34·2 ± 2·5	36·8 ± 3·7 (n = 21)
2	0	27·0 ± 1·4		35·5 ± 2·2 (n = 20)
	10	27·2 ± 1·0		35·0 ± 1·9 (n = 22)
	100	27·9 ± 1·4		35.3 ± 2·7 (n = 20)
3	0	23·8 ± 0·8		34·6 ± 2·9 (n = 20)
	10	23·6 ± 0·9		35·0 ± 2·0 (n = 22)
	100	23·6 ± 1·1		34·0 ± 2·0 (n = 22)

Mice were given the indicated intragastric doses of ebselen for 21 days. Results are means ± s.d. of results for the numbers of mice indicated in parentheses.

Table 4. Susceptibility to infections of mice treated with ebselen for 21 days.

Experiment	Microorganism	Inoculum (cells/mouse)	Survivors Ebselen ($mg\,kg^{-1}$)		
			0	10	100
1	*C. albicans*	10^8	12/20	12/21	13/21
2	*P. aeruginosa*	1.5×10^7	18/20	20/22	18/20
3	*S. aureus*	5×10^8	18/20	20/22	22/22

Infection with P. aeruginosa

Mice were similarly grouped and administered ebselen as described above. In a preliminary experiment, 0 of 3, 3 of 4, and 1 of 2 untreated mice died 48 h after the intraperitoneal injection of 10^7, 3×10^7 and 10^8 *P. aeruginosa* cells per mouse, respectively. Ebselen-treated mice were injected intraperitoneally with a semilethal number of *P. aeruginosa* cells ($1{\cdot}5 \times 10^7$ cells) on day 21. Eighteen of 20 control mice survived after 48 h. The rate of survival did not differ among the three groups (Table 4, experiment 2).

Infection with S. aureus

Mice were similarly grouped and treated with ebselen as described above. In a preliminary experiment, 1 of 3, 0 of 3, and 3 of 4 untreated mice died 48 h after the intraperitoneal injection of 2×10^8, $6{\cdot}7 \times 10^8$ and 2×10^9 methicillin-resistant *S. aureus* (MRSA) cells per mouse, respectively. Ebselen-treated mice were injected intraperitoneally with a semilethal number of MRSA cells (5×10^8 cells) on day 21. Eighteen of 20 control mice survived after 48 h. The rate of survival did not greatly differ among the three groups (Table 4, experiment 3).

Discussion

The anti-inflammatory effects of ebselen have been demonstrated in ischaemia reperfusion injury in myocardial infarction (Hoshida et al 1994) and in the cerebral cortex (Matsui et al 1990), in haemorrhagic pancreatitis (Niederau et al 1991), in injury to the gastric mucosa (Kurebayashi et al 1989), in acute liver failure (Akasaki et al 1989), in arthritis (Schalkwijk et al 1986) and in lung oedema (Cotgreave et al 1988) in various animal models. The anti-inflammatory action of ebselen is considered to be due to its activity to reduce organic hydroperoxides as well as reactive oxygen metabolites produced by inflammatory cells (Parnham & Graf 1987). As shown in Table 1, higher concentrations of ebselen (30 μM) greatly inhibited the candidacidal activity of human neutrophils and macrophage-like cells, a result that can be partially explained by the fact that ebselen inhibits PMA-mediated production of H_2O_2 over the same range of concentrations (Table 2). Therefore, long-term administration of ebselen as an anti-inflammatory agent might be considered likely to produce an immunocompromised host. In this study, ebselen was administered intragastrically by cannulation every day for three weeks to young male mice and then these mice were infected with bacteria and *Candida*. Ebselen up to $100\,mg\,kg^{-1}$, which exhibits anti-inflammatory action in animal models described above, hardly affected the increase in body weight during the 21-day period (Table 3), reflecting the low toxicity of ebselen (Parnham & Graf 1987). Furthermore, these mice did not become susceptible to *C. albicans*, *P. aeruginosa* or MRSA (Table 4). Thus, it appeared that mice remained able to fight infections during the administration of a high concentration of ebselen. Ebselen is rapidly metabolized to a selenoglucuronide and a selenomethyl compound after oral administration in man and animals (Fischer et al 1988; Terlinden et al 1988). Intact ebselen has never been found in human plasma. If ebselen is also degraded in mice, the neutrophils as well as the macrophages in mice given oral ebselen might never be exposed to the sufficiently high concentrations of ebselen that might impair their microbicidal activity. Nevertheless, oral ebselen does have an anti-inflammatory effect in a variety of animal models of inflammation. Therefore, a very low concentration of intact ebselen might have anti-inflammatory actions in-vivo, or some metabolite of ebselen might be the actual anti-inflammatory agent.

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