

Anti-inflammatory properties of ebselen in a model of sephadex-induced lung inflammation

M.G. Belvisi, E-B. Haddad, C. Battram, M. Birrell, M. Foster, S. Webber

Anti-inflammatory properties of ebselen in a model of sephadex-induced lung inflammation. M.G. Belvisi, E-B. Haddad, C. Battram, M. Birrell, M. Foster, S. Webber. ©ERS Journals Ltd 2000.

ABSTRACT: Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one), is a seleno-organic compound which protects tissues against oxidative stress. Furthermore, recent data has suggested that this compound possesses a range of anti-inflammatory properties.

In this study the authors have investigated the effects of ebselen on Sephadex-induced lung oedema and bronchoalveolar lavage (BAL) tumour necrosis factor (TNF)- α and endothelin(ET)-1 levels in rats.

Sephadex administration induced lung oedema which was accompanied by an increase in BAL TNF- α and ET-1 levels. Ebselen administration (1–30 mg·kg⁻¹, *i.p.* at 0, 4 and 12 h post Sephadex) significantly inhibited lung oedema (dose that produced 50% of the maximum inhibition of lung oedema 4.6 mg·kg⁻¹) and BAL TNF- α levels in a dose-related manner with no effect on ET-1 levels.

These data suggest that ebselen may be a useful therapy in lung pathologies in which bronchiolar inflammation is a feature.

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Aventis Pharma, Pharmacology Dept, Dagenham Research Centre, Dagenham, Essex, UK.

Correspondence: M.G. Belvisi, Pharmacology Dept, Aventis Pharma, Dagenham Research Centre, Rainham Road South, Dagenham, Essex RM10 7XS, UK. Fax: 44 1819192497

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The Sephadex model of lung oedema in the rat is a model of acute alveolitis and bronchiolitis leading to inflammatory cell infiltration and interstitial oedema which appears to parallel many of the pathophysiological features associated with human interstitial lung diseases [1]. This model has been used previously to demonstrate the anti-inflammatory properties of compounds such as glucocorticosteroids based upon the modulation of lung oedema [2].

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is a selenium-containing organic compound which has been found to protect tissue against oxidative attack by mimicking glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase [3]. In addition, ebselen possesses a range of anti-inflammatory activities including inhibition of the enzymes 5-lipoxygenase, inducible nitric oxide synthase, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and inactivation of leukotriene B₄ to its biologically inactive 6-trans isomer [4]. In view of its interesting anti-inflammatory profile in a variety of animal models of inflammation [1, 5], the authors investigated its effects in the Sephadex model of lung oedema. In addition, the effect of ebselen was examined on tumour necrosis factor (TNF)- α and endothelin(ET)-1 levels in the bronchoalveolar lavage (BAL) fluid from Sephadex-treated rats since these cytokines have been postulated to have a role in inflammatory lung disease [6–9].

Materials and methods

Animals

Male, Sprague-Dawley rats (350 g) were purchased from Harlan-Olac (Bicester, Oxfordshire, UK) and housed for 1 week before initiating experiments. Food and water were supplied *ad libitum*. Experiments were performed in accordance with the UK Home Office guidelines for ani-

mal welfare based on the Animals (Scientific Procedures) Act 1986 [10] and following approval from the Aventis Pharma Animal Care and Use Committee.

Methods

Rats were dosed intratracheally (*i.t.*) with vehicle (saline) or Sephadex beads (5 mg·kg⁻¹) in a dose volume of 1 mL·kg⁻¹ under halothane anaesthesia (4% in oxygen for 3 min). Ebselen (1–30 mg·kg⁻¹) or vehicle (25% dimethyl sulfoxide (DMSO), 75% polyethylene glycol (PEG) 200) was administered intraperitoneally (*i.p.*) following Sephadex administration (0 h) and then at 4 and 12 h post Sephadex in a dose volume of 0.5 mL·kg⁻¹. The dosing regimen was determined from a previous study [1] which demonstrated preliminary data describing the inhibitory activity of a single dose of ebselen (10 mg·kg⁻¹, *i.p.*) on sephadex-induced lung oedema.

Measurement of lung oedema

Rats were sacrificed 24 h post-sephadex with Euthatal (1 mL·kg⁻¹, *i.p.*), the heart and lungs removed *en bloc*, and the lung wet weights determined and expressed per 100 g initial body weight. Percentage inhibition of oedema (Sephadex *i.t.*/vehicle *i.p.* control) was then determined for each treatment group. A dose-response curve was generated, a sigmoidal fit obtained for the data and the dose that produced 50% of the maximum inhibition of lung oedema (ED₅₀) was calculated.

Measurement of cytokine production in the bronchoalveolar lavage fluid

Rats were sacrificed 24 h post-Sephadex with Euthatal (1 mL·kg⁻¹, *i.p.*) and lavaged (for 30 s at room temperature)

at 1 mL·kg⁻¹ body weight with Roswell Park Memorial Institute medium (RPMI)/glutamate plus 10% foetal calf serum (FCS). This was repeated once and the two samples pooled. The BAL fluid sample was then centrifuged at 800 × g for 10 min and the supernatant frozen for later analysis. BAL fluid interleukin(IL)-1β, TNF-α and ET-1 levels were determined by enzyme linked immunosorbent assay (ELISA).

Euthatal and Halothane were obtained from Rhône Mérieux (Harlow, Essex, UK) and RPMI from GIBCO, (Paisley, Strathclyde, Scotland, UK), Sephadex G-200 was purchased from Pharmacia (Uppsala, Sweden). DMSO and PEG 200 were purchased from Sigma (Poole, Dorset, UK). Ebselen (2-phenyl-1,2-benzisoxazol-3(2H)-one) was synthesized by the Chemistry Department of Rhône-Poulenc Rorer (Vitry, France). Rat IL-1β and TNF-α ELISA kits were purchased from Life Screen Ltd (Watford, UK) and Genzyme (West Malling, UK), respectively. Human ET-1 ELISA kits were purchased from R&D Systems (Abingdon, UK).

Analysis

All values presented are mean±SEM from n=8 rats per group. The percentage inhibition of oedema (compared to the Sephadex administered, vehicle-treated group) was determined for each Ebselen-treated group. The dose-response curve for inhibition of lung oedema by Ebselen was calculated by least squares, nonlinear iterative regression with the "PRISM" curve fitting programme (Graphpad Instat software programme, San Diego, CA, USA). An ED50 value was subsequently interpolated from a curve of best fit. The data were analysed using the Kruskal-Wallis non-parametric test with a correction for multiple comparison's using Dunnett's critical values. A p-value of less than 0.05 was considered to be statistically significant.

Results

Lung oedema

Sephadex instillation alone evoked a significant oedema, 38.5% (p<0.01), which was reduced to 26.3% in vehicle-treated animals. This inhibition of oedema in the vehicle-treated group was not significant. Ebselen evoked a dose-dependent inhibition of lung oedema (ED50 of 4.6 mg·kg⁻¹) when compared to lung wet weights from Sephadex instilled, vehicle-treated animals (fig. 1).

Cytokine production in the bronchoalveolar lavage fluid

There were no detectable levels of IL-1β, TNF-α or ET-1 in the vehicle treated group. There were no detectable levels of IL-1β in BAL from Sephadex-treated rats but there was a significant increase in BAL TNF-α (fig. 2) and ET-1 levels (vehicle treated group, 0.043±0.02 pg·mL⁻¹; Sephadex-treated group, 1.35±0.29 pg·mL⁻¹, p<0.001) compared to the vehicle-treated group. Ebselen dose-dependently reduced BAL TNF-α levels when compared to the Sephadex/vehicle control group. This effect was significant at 10 and 30 mg·kg⁻¹ (fig. 2). ET-1 levels were not altered by ebselen (0.3–30 mg·kg⁻¹).

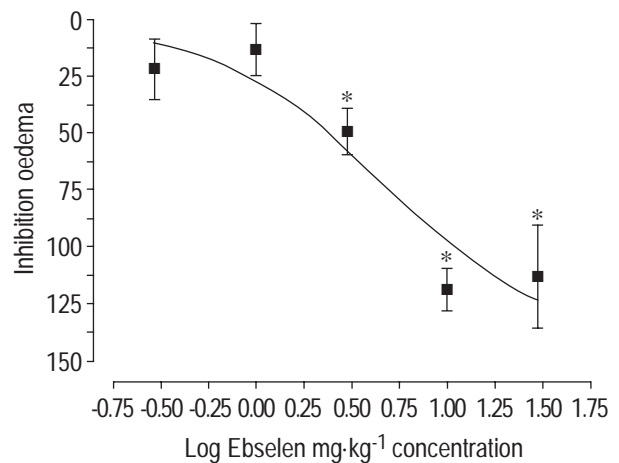


Fig. 1. – Inhibitory effect of ebselen (0.3–30 mg·kg⁻¹, administered immediately after Sephadex instillation and then at 4 and 12 h post Sephadex, *i.p.* on Sephadex-induced lung oedema in the rat. Points represent mean±SEM of eight rats.

Discussion

In this study the authors have investigated the effect of ebselen on Sephadex-induced lung oedema and BAL TNF-α levels. Sephadex particles induce lung oedema 24 h post-instillation. This effect was completely inhibited in a dose-dependent fashion by ebselen with an ED50 of 4.6 mg·kg⁻¹ and confirms previous preliminary findings [1] in which COTGREAVE *et al.* [1] demonstrated 98% inhibition of Sephadex-induced lung oedema at a single dose of 10 mg·kg⁻¹. The data presented here takes these observations further by establishing that the inhibitory effect of ebselen is dose-related. In addition, the authors have determined the potency and maximal efficacy of this compound and attempted to elucidate the mechanism of action of ebselen in this model.

In addition, the authors investigated the effect of ebselen on TNF-α levels in the BAL fluid from Sephadex-treated rats since this cytokine has been postulated to have a role in

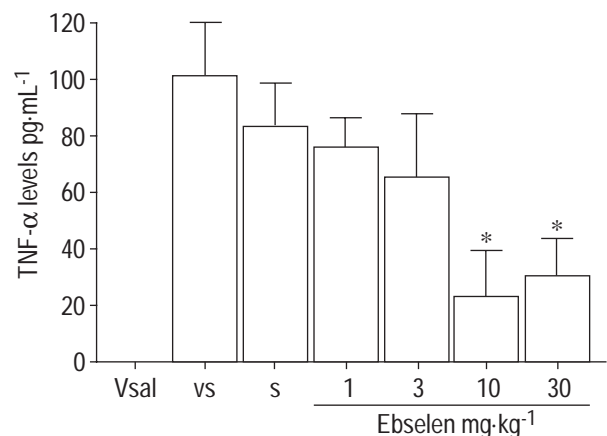


Fig. 2. – Effect of ebselen (1–30 mg·kg⁻¹) on Sephadex-induced tumour necrosis factor (TNF)-α levels in bronchoalveolar lavage (BAL) fluid. The effects of vehicle on saline (Vsal)- and sephadex(vs)-induced TNF-α levels as well as the effect of sephadex alone (s) on TNF-α levels in BAL fluid are also shown. BAL fluid supernatants were assayed for TNF-α levels by enzyme linked immunosorbent assay (ELISA). Data are mean±SEM of eight rats.

an animal model of antigen-induced oedema [10] and in inflammatory lung disease [6]. Indeed, TNF- α levels are increased in several inflammatory diseases including asthma and chronic obstructive pulmonary diseases [7, 11]. It has also previously been demonstrated that Sephadex particles increase TNF- α messenger ribonucleic acid (mRNA) and protein expression in lung epithelial cells, lung granulomas and BAL cells [12]. Interestingly, IL-1 β and TNF- α have been shown to induce an increase in prepro-ET-1 mRNA and ET-1 peptide expression in cultured pulmonary endothelial cells of rats [13]. In addition, Sephadex instillation evokes an increase in ET in the BAL fluid and the mixed ETA/B receptor antagonist, Bosentan, inhibits the cellular inflammatory response elicited by Sephadex [14]. In this study the authors have explored the hypothesis that Sephadex increases the expression of TNF- α leading to an increase in ET-1 levels which is involved in the production of Sephadex-induced lung oedema. However, the data presented demonstrate that ebselen dose-dependently reduced TNF- α , but not ET-1, levels in the BAL fluid of Sephadex-treated rats. These data suggest that the anti-inflammatory action of ebselen may be due to its inhibitory action on TNF- α release and that the increased levels of ET-1 following Sephadex instillation are not associated with the production of Sephadex-induced lung oedema.

COTGREAVE *et al.* [1] have also demonstrated that ebselen inhibits infiltration of eosinophils, lymphocytes and basophils into the airway lumen in response to Sephadex whilst leaving the macrophage and neutrophil populations largely unchanged. This profile of biological activity would appear to be ideal for a compound targeted at treating lung inflammatory diseases as ebselen possesses the required anti-inflammatory activity whilst allowing continued protection of the lung from opportunistic infections by the presence of macrophages and neutrophils.

In conclusion, these data demonstrate that ebselen possesses anti-inflammatory activity in this rat model of lung inflammation which may be related to inhibition of tumour necrosis factor- α production. These results also suggest that this drug may be useful in a range of lung pathologies in which bronchiolar inflammation is a feature.

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