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

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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.

Keywords: Airway inflammation lung oedema sephadex tumour necrosis factor-α

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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 animal 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-Sepahadex 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 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, 0.04±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).

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
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-1β 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 ebseleone 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 ebseleone 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 ebseleone 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 ebseleone 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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**References**

1. Cotgreave IA, Johansson U, Westergren G, Moldeus PW,