The anti-inflammatory activity of Ebselen but not thiols in experimental alveolitis and bronchiolitis

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Abstract

This paper describes the effects of the thiol compounds glutathione and N-acetylcysteine and the seleno-organic agent Ebselen on the development of Sephadex-induced lung edema and cell infiltration in the rat. Neither thiol had any effect upon the development of the edema when administered in large, repeated doses. In contrast, when Ebselen was co-administered with the thiols, there was a dose-dependent inhibition of the development of the edema, but lung weights could not be returned to normal values. However, when the thiols were omitted and Ebselen was administered alone, the development of the edema was totally blocked. In addition, in Ebselen-only treated animals there was a selective inhibition of the infiltration of lymphocytes, basophils and eosinophils into the lung lumen without affecting the populations of macrophages and neutrophils. Again, the Ebselen-induced effect was reduced by coadministration of either thiol. These findings are discussed in terms of the potential mechanism of action of Ebselen in vivo and of the possibility of Ebselen being of therapeutic potential in cases of diffuse pulmonary inflammation in humans.

Introduction

One of the most characteristic symptoms of the inflammatory state is the induction of vascular permeability changes, leakage of plasma exudate into the interstitium and resultant edema [1]. Several inflammatory principles are thought to be involved in the induction of edema [2]. Central to these are the reactive oxygen species ($\text{H}_2\text{O}_2$, $\text{O}_2^-$ and $\cdot\text{OH}$) produced by inflammatory cells such as granulocytes and macrophages [3–5]. These species may effect permeability changes by causing localised cytotoxicity within the vascular endothelium [4, 6] or through stimulation of lipid peroxidation and arachidonic acid metabolism to LTs via the activity of 5'-lipoxygenase. These arachidonic acid metabolites have both a direct effect upon vascular permeability ($\text{LTC}_4$) and contribute to the recruitment of further inflammatory cells to the site ($\text{LTB}_4$) [7]. However, the relative importance of these principles as well as their localisations is still uncertain.

Abbreviations: $\text{O}_2^-$, Superoxide anion; $\text{H}_2\text{O}_2$, Hydrogen peroxide; $\cdot\text{OH}$, Hydroxyl radical; PMN, Polymorphonuclear leukocytes; ACU, Alveolar-capillary unit; GSH, Glutathione; NAC, N-acetylcysteine; Ebselen, 2-phenyl-1,2-benzisoselenazol-3(2H)-one; DMSO, Dimethylsulfoxide; PEG, Polyethylene glycol; GSH-px, Glutathione peroxidase; DMTU, Dimethylthiourea; LT, Leukotriene.

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We have developed a model of acute alveolitis and broncheolitis in the rat leading to inflammatory cell infiltration of the ACU (i.e. interstitially and intralumenally) and interstitial edema [8], events symptomatic of a variety of human interstitial lung diseases (e.g. alveolitis) [9-11]. The model is based upon the use of intratracheally instilled Sephadex beads which provoke inflammation via at least two mechanisms, their particulate state is a non-specific trigger and furthermore the beads are composed of crosslinked dextran. Rats have an endogenous hypersensitivity to dextran. This model has been used in the development of anti-inflammatory principles based upon the modulation of the pro-edemic principles detailed above. Thus, we have demonstrated anti-edemic efficacy of glucocorticosteroids, agents known to interfere with the metabolism of arachidonic acid to LTs [12]. In this paper, two “novel” anti-inflammatory principles have been tested in the Sephadex model. The first of these is represented by NAC and GSH. NAC is a thiol-containing drug used clinically in the treatment a variety of airways conditions [13]. Recent attention has been drawn to the pneumoprotective potential of NAC as it is known to directly detoxify reactive oxidants through the chemical reactivity of its thiol group and indirectly interact with such species through its metabolism to the endogenous cytoprotective tripeptide GSH [14]. Indeed, recently, NAC has demonstrated efficacy in pulmonary oxygen toxicity [15], which is thought to be largely attributable to increased oxidant burden in the lungs. Additionally, we have tested the seleno-organic compound Ebselen [16] alone and in combination with these thiols. Ebselen may potentially interact with pro-edemic principles at a variety of levels. Ebselen is known to inhibit lipid peroxidation through its activity as an anti-oxidant [17, 18] and to enhance the reaction of GSH and NAC with H2O2 [17] and other hydroperoxides through its catalytic GSH-px-like activity [19]. Additionally, Ebselen interacts with arachidonic acid metabolism at a variety of levels in vitro. An inhibition of 5'-lipoxygenase has been implicated through in vitro studies of the utilization of arachidonic acid by leukocytes [20]. Ebselen has also been shown to catalyze the isomerization of LTB4 to the inactive 6-trans isomer [21]. Ebselen has also previously been shown to posses anti-inflammatory activity in a variety of other inflammation models [22, 23].

Materials and methods

Chemicals and drugs

Ebselen was the kind gift of A. Nattermann, Cie GmbH, Köln, FRG. GSH, DMSO and PEG were purchased from Sigma Chemical Co, St. Louis, USA. NAC was supplied by AB Draco, Lund, Sweden. Sephadex (G-200 superfine) was obtained from Pharmacia, Uppsala, Sweden.

Animals and dosing regimes

Male Sprague-Dawley rats (250 g) were acclimatized to the experimental environment for at least 3 days prior to use, weighed and segregated into various control and test groups. The animals received Sephadex beads by intratracheal instillation (iti) as described previously [8, 24]. Drug solutions were prepared as follows: GSH and NAC were dissolved to 100 mg/ml in physiological saline with pH adjustment to 7.4 with 10 M NaOH. Ebselen was initially dissolved in DMSO and then mixed 1:4 with PEG giving a final Ebselen concentration of 25 mg/ml. Animals received the thiols (400 mg/kg) i.v. through a tail vein. Ebselen was dosed i.p. (10 mg/kg). Animals received the drugs under ether anesthesia as outlined in Table 1. Concurrent controls were performed for the effects of the dosing vehicles on lung edema and cell infiltration. Twentyfour hours after Sephadex instillation animals were reweighed, sacrificed with a lethal dose of Mebumal (ACO Läkemedel, Sweden) and the lungs either lavaged for the intralumenal cell content [25] or removed and weighed wet for the assessment of edema.

Differential cell counting

Total lavage cells were counted in a Bürker chamber following centrifugation of lavage fluid (500×g, 10’, 4°C). The differential cell count (macrophages, basophils, lymphocytes, neutrophils and eosinophils) in the lavage was assessed on Cytospin preparations stained with May-Grünwald and Giemsa stains.