TROLOX EFFECT IN A MICE MODEL OF TOLUENE DIISOCYANATE -INDUCED ASTHMA

O. Perseca¹, R. Orasan², Alina Elena Parvu³, M.A. Taulescu⁴, R. Moldovan²

¹OCCUPATIONAL MEDICINE, REGIONAL PUBLIC HEALTH CENTER CLUJ-NAPOCA, ROMANIA; ²PHYSIOLOGY, ³PATHOPHYSIOLOGY, - "IULIU HATIEGANU" UNIVERSITY OF MEDICINE AND PHARMACY CLUJ-NAPOCA, ROMANIA; ⁴PATHOLOGY, UNIVERSITY OF AGRICULTURAL AND VETERINARY SCIENCES, CLUJ-NAPOCA, ROMANIA

Summary

The mechanisms of occupational asthma are not completely known. The aim of the study was to evaluate the effect of an antioxidant substance, Trolox, on the histopathological changes associated to an experimental mice Toluene-2,4-diisocyanate (TDI)-induced asthma. The results showed that by reducing oxidative stress, Trolox reduced the inflammatory infiltrate in the peribronchial and perivascular regions.

Keywords: occupational asthma, TDI, oxidative stress, trolox

parvualinaelena@yahoo.com

Introduction

Occupational asthma is the most common work-related lung disease. It may be induced by exposure to low molecular weight agents such as diisocyanates, acid anhydrides, reactive dyes, and many other chemicals (Vanoirbeek et al., 2004). Diisocyanates are low molecular weight chemicals widely used in industry for the production of polyurethane foams, varnish, paint, and isolation material (Bello et al., 2007, Jones et al., 2006, Liu and Wisnewski 2003, Mapp et al., 2005, Wisnewski and Redlich, 2001, Vanoirbeek et al., 2009). They are one of the leading causes of occupational asthma worldwide. Experimental animal models of diisocyanate occupational asthma have demonstrated an immunological basis for the disease. Mice can be sensitized by dermal or respiratory exposure, suggesting that either the exposure route may be important in the workplace. Murine model for occupational asthma generates both inflammatory and immune mediators similar to those occurring in TDI-induced asthma in humans (Matheson et al., 2001).

It was found that isocyanate-induced lung disease, is an oxidant stress-dependent pulmonary inflammation. Diisocyanates can react with -OH, -SH, and -NH₂ groups of endogenous proteins (Vandenplas et al., 1993). It was demonstrated that diisocyanates are able to bind to glutathione in the skin or mucosal surfaces (Vanoirbeek et al., 2009). Glutathione (GSH), one of the major anti-oxidants of the lung, has been linked to the response to isocyanate exposure. Experimental data suggest that airway GSH may help prevent the development of allergic sensitization and asthma (Wisnewski et al., 2005).

Considering the data about isocyanate-induced asthma and oxidative stress, the aim of the study was to evaluate Trolox effect, as an antioxidant, on the morphological pulmonary changes in an experimental mouse model of TDI-induced asthma.

Material and methods

Chemicals

Toluene-2,4-diisocyanate (98%; Fluka, CAS 584-84-9) was obtained from Aldrich Chemical Co. (Taufkirchen, Germany). The vehicle (AOO) used to dissolve the TDI consisted of a mixture of 2 volumes of acetone (Borealis AG, Viena, Austria).
Austria) and 3 volumes of olive oil (extra virgin, Pietro Coricelli S.p.a., Spoleto, Italy). Concentrations of TDI are given as percentages (v/v).

**Animals**

Male mice (approximately 20 g, 5-6 weeks old) were obtained from the Animal Facility of Iuliu Hatieganu University of Medicine and Pharmacy (Cluj Napoca, Romania). The mice were housed in a conventional animal house with 12-h dark/light cycles, and received water and pelleted food ad libitum. All experimental procedures were approved by the local Ethical Committee for Animal Experiments.

**Groups of animals and treatment protocol**

As previously described, on days 1, 2, 3 and 7, the animals received a dermal application (20 µl) with 0.3% TDI, or vehicle (AOO, 2:3) on the dorsum of both ears. On day 10, they received, under light diethyl ether anesthesia, an intranasal instillation (10 µl/nostril) of 0.1% TDI (challenge), or vehicle (AOO, 1:4). Mice were deeply anesthetized with pentobarbital (90 mg/kg i.p.) and sacrificed 24 hours after the challenge (Figure 1). In all experiments, treatment with TDI is indicated as 1, and treatment with vehicle is indicated as 0. Thus, there were four study groups: group 1/1, mice that received dermal sensitization with TDI and an intranasal challenge with TDI; control group 0/0 mice that received the AOO vehicle on all occasions; group 0/1 mice that received dermal sensitization with AOO vehicle and an intranasal challenge with TDI; group 1/1+Trolox, mice that received TDI on all occasions and Trolox treatment; group 0/0+Trolox, mice that received AOO and Trolox; group 0/1+Trolox, mice that received dermal sensitization with AOO, an intranasal challenge with TDI and Trolox treatment (Tarkowski et al., 2007). Each group consisted of 12 animals. Trolox (20 mg/kg/day i.p) was administrated from day one to day 10 (McClung et al., 2007). Trolox treatment was administered to a 0/0 group in order to determine the independent effects of Trolox on non-sensitized animals.

![Figure 1](image)

**Histopatologic examination**

Twenty-four hours after the airway challenge, mice were sacrificed and lungs were collected. Tissues were immersed in 10% neutral buffered formalin, embedded into paraffin, serially sectioned, and stained with hematoxylin eosin for blinded histopathologic assessment (Matheson et al., 2002, Matheson et al., 2005).

The histopathologic grading system was expressed on a 0–3 scale (Curtis et al., 1991) for each animal. The main
histological features that were graded were peribronchial, perivascular and interstitial inflammatory infiltration. Histologic grading criteria were: Grade 0 - no inflammation; Grade 1 - rare inflammatory cells, and they are not arranged in bands or cords; Grade 2 - inflammatory cells arranged around bronchi or vessels, forming fine bands (between 1 and 5 cells per row); Grade 3 - inflammatory cells arranged around bronchi or vessels, forming thick bands around bronchi or vessels. Each animal result was expresses as mean and SE of five sections from each lung.

**Data analysis.** All data are presented as means and standard error (SE). The groups were compared with a one-way ANOVA test. A level of p<0.05 was considered significant.

**Results and discussions**

At group 1/1 there was bronchiolar and alveolar wall thickening due to bronchiolar subepithelial fibrosis and hypertrophy/hyperplasia of the smooth muscle. Perivascular and in the interstitial tissue there was a rich inflammatory infiltrate with mononuclear, neutrophils and eosinophils. Epithelial hyperplasia was associated with an increased number of Goblet cells (Figure 2).

![Figure 2. Representative photomicrographs of lungs of mice from group 1/1. Subepithelial bronchiolar fibrosis, epithelial hyperplasia, peribronchiolar inflammatory infiltrate with mononuclear cells, eosinophils and neutrophils, intramural interalveolar, and interstitial, pulmonary hemorrhage; HEx10; x200.](image)

The isocyanate-induced asthma include airway inflammation, involving the presence of activated T cells, eosinophils, neutrophils, and mast cells (Matheson *et al.*, 2005).

Some studies found at the histological evaluation just a minor influx of eosinophils around the blood vessels in the lungs of mice sensitized and challenged by TDI, and no significant histological change in those treated with only with vehicle (Vanoirbeek *et al.*, 2004).

Using different inbred mouse strains for models of chemical-induced asthma, in was demonstrated that the genetic background has an important influence on the phenotypical outcome of TDI-induced asthma (De Vooght *et al.*, 2010). This may be an explanation for the more severe histopathological response to TDI in our animals.

The influx of neutrophils rather than eosinophils is not contradictory with allergic asthma. The nature of the pulmonary inflammation in asthma is presumably dependent on the time course of the disease and the pattern of the exposure. Neutrophilic inflammation has also been observed in TDI asthmatics (Vanoirbeek *et al.*, 2009).

At group 0/0 no significant changes were found (Figure 3).

![Figure 3. Representative photomicrographs of lungs of mice from group 0/0. Normal histological aspect. HEx4, x 500.](image)
Figure 4. Representative photomicrographs of lungs of mice from group 0/1. Diffuse pulmonary congestion, hemorrhage per diapedesis, subepiteliel bronchiolar fibrosis, peribronchiolar inflammatory infiltrate with neutrophils and mononuclear cells. HEx10, x200.

Trolox is a peroxyl radical scavenger that has previously been shown to rapidly penetrate cell membranes and be more effective as an antioxidant than its parent compound, vitamin E (Sagach et al., 2002). The primary protective effect of Trolox appears to be based on its capacity to inhibit membrane lipid peroxidations and protein carbonylation (Bizzozero et al., 2007, McClung et al., 2007). Trolox treatment reduced the inflammatory infiltrate in the groups 1/1 (Figure 5) and 0/1 (Figure 6).

Figure 5. Representative photomicrographs of lungs of mice from group 1/1+Trolox. The Trolox treatment reduced the perivascular and peribronchiolar inflammatory infiltrate. HEx20, x100.

Figure 6. Representative photomicrographs of lungs of mice from group 0/1. Trolox treatment reduced the perivascular inflammatory infiltrate. HEx200, x100.

Quantification of inflammatory infiltrate in lung parenchyma showed a higher infiltrate at 1/1 group, and a smoother infiltrate at group 0/1 (p<0.01). At 1/1 animals Trolox reduced the peribronchial and perivascular inflammatory infiltrate. At 0/1 animals Trolox decreased only the perivascular infiltrate. At 0/0 there was no change after Trolox association (Table 1).

Table 1: Inflammatory infiltrate grading

<table>
<thead>
<tr>
<th>Groups</th>
<th>peribronchial infiltrate</th>
<th>perivascular infiltrate</th>
<th>interstitial infiltrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1</td>
<td>3*</td>
<td>3*</td>
<td>3*</td>
</tr>
<tr>
<td>0/1</td>
<td>2*#</td>
<td>2*#</td>
<td>3*</td>
</tr>
<tr>
<td>0/0</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>1/1+ Trolox</td>
<td>2*#</td>
<td>1*#</td>
<td>3*</td>
</tr>
<tr>
<td>0/1+Trolox</td>
<td>2*#</td>
<td>1*#</td>
<td>3*</td>
</tr>
<tr>
<td>0/0+Trolox</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

Each value represents a mean ± SE (n = 12).

* Significantly different from the 1/1 group (p < 0.05).

# Significantly different from 0/0 group (p < 0.05).
Conclusions

The present study proved that the pulmonary inflammation observed in the model of TDI-induced asthma, depends on the oxidative stress, since Trolox, an antioxidant substance, reduced the inflammatory infiltrate.

References


