Increased lung to blood passage of polyethylene glycols after intratracheal instillation of ferritin and asbestos fibres in the rat

H.G. Folkesson*, P. Leanderson**, B.R. Weström*, C. Tagesson**


ABSTRACT: Urinary recovery of intratracheally instilled polyethylene glycol polymers (PEG:s) in the molecular weight range 722-1294 Da (PEG 1000) was studied under normal conditions and during experimentally induced lung damage in rats. The urinary PEG recoveries were between 30-60% under normal conditions, with a selectivity for smaller PEG:s. No significant differences in the urinary PEG molecular weight profiles were found between 30 days old and adult rats; i.e. they had similar PEG 1162/810 (molecular weights) urinary recovery ratios (0.78±0.25 and 0.69±0.27, respectively, p<0.05).

In rats instilled with PEG 1000 and ferritin (5 mg·kg⁻¹ body weight), the urinary recovery was increased for PEG:s with molecular weights greater than 1030 Da; i.e. a higher PEG 1162/810 recovery ratio (1.44±0.58, p<0.01) was obtained. Rats instilled with PEG 1000 and crocidolite asbestos fibres (5 mg·kg⁻¹ body weight) showed higher urinary recoveries for PEG:s greater than 854 Da, resulting in a higher PEG 1162/810 ratio (1.47±0.59, p<0.01). By adding the iron-chelator, deferoxamin, to the crocidolite-instillate, the urinary recoveries and the PEG 1162/810 ratio (0.97±0.47) were reduced, indicating a restored molecular weight selectivity of the lung.

Thus, in rats, PEG 1000 passes via the respiratory tract in large amounts which is dependent on the molecular weight. This passage was increased after ferritin- or crocidolite instillation, indicating that the barrier function of the respiratory tract was impaired due to local tissue damage, and that iron may play an important role in this.


A variety of molecules are able to pass across the epithelium of the respiratory tract and into the blood stream [1-3]. Proteins and peptides can be traced intact in the blood after passage through the broncho-alveolar epithelium [1, 3, 4] and depending on the molecular weight this passage varies between 1.5-20% of given dose in the rat [3]. Small inert molecules like technetium-labelled diethylenetriamine pentaacetic acid (⁹⁹ᵐTc-DTPA) and low molecular weight polyethylene glycols (PEG:s) have also been used as markers for assessing the molecular passage during healthy and inflammatory conditions in the lungs. By using ⁹⁹ᵐTc-DTPA it has been shown that clinically stable asthmatics do not show increased lung permeability [5] while other injurious conditions in the lungs resulted in an increased permeability [6, 7]. LINDHOLM et al. [8] showed that high concentrations of lysophosphatidylcholine (lyso-PC) induced an inflammation and increased the airway epithelial permeability of PEG in the molecular weight range 502-722 Da. In the same study it was shown that low concentrations of lyso-PC increased the capillary permeability for the PEG.

In a study where lung inflammation was induced by intratracheal instillation of ferritin, increased passage of bovine IgG and bovine serum albumin was obtained, while the passage of the nona-peptide, 1-deamino-lysine-8-D-arginine vasopressin (dDAVP), was less affected [9].

The maturity of the pulmonary epithelium may also influence the passage of different-sized molecules [2, 3]. Newborn rats show an enhanced permeability for low molecular weight compounds, such as mannitol, as compared to adult rats [2], while macromolecular compounds, such as proteins, show a more complex pattern with a decreasing molecular weight selectivity of the pulmonary epithelium with increasing age of the rat [3].

The present study was undertaken in order to investigate the lung passage of the inert marker PEG 1000, consisting of different-sized ethylene glycol polymers ranging from 722-1294 Da, after intratracheal instillation in the rat. The study was conducted both under normal healthy conditions in adult and young rats and during experimentally induced lung damage by...
intrathoracic instillation of two different iron-containing agents; ferritin and crocidolite asbestos fibres. Occupational exposure to asbestos has been shown to cause several lung disorders such as asbestosis, mesothelioma and lung cancer [10]. Although several studies have been performed over the last 60 yrs, little is known about the mechanism by which asbestos fibres cause the mentioned lung disorders. However, it has recently been suggested that iron-catalyzed reactions can be responsible for the effects [11].

Materials and Methods

Experimental animals

Sprague Dawley rats (Alab, Sollentuna, Sweden) of both sexes were used; either 30 days old, weighing 70–100 g, or 100–120 days old (adult) weighing 250–400 g. The rats were kept on chopped wood beddings in polycarbonate cages under a 12 h day:12 h night rhythm at 20±2°C (sr) and at a relative humidity of 50±10%. The rats had free access to a standard rat chow (R3, Ewos, Södertälje, Sweden) and tap water.

To study the influence of lung injury on the passage of molecules, rats were intratracheally instilled with the PEG 1000 solution together with 5 mg ferritin·kg⁻¹ body weight (F-4503, Sigma Chemical Co., St. Louis, MO, USA). This dose of ferritin has previously been shown to give an acute lung inflammation including oedema [9]. Another group of rats were instilled with PEG 1000 together with 5 mg crocidolite asbestos fibres·kg⁻¹ body weight. The crocidolite asbestos was from the International Union against Cancer Conference (UICC) Reference Standard Samples [13] and was kindly supplied by H. Pezerat, Paris, France. In one series of experiments, the role of iron in the crocidolite was studied by adding the iron chelator desferrioxamine (250 mg·kg⁻¹ body weight,

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Lavage volume ml</th>
<th>Total protein mg</th>
<th>Total cell number x10⁶</th>
<th>Macrophages x10⁶</th>
<th>Neutrophils x10⁶</th>
<th>Mitoses %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>6</td>
<td>33.6±6.6</td>
<td>7.5±0.9</td>
<td>3.5±2.1</td>
<td>3.4±2.0</td>
<td>0.1±0.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>PEG 1000</td>
<td>6</td>
<td>36.1±2.5</td>
<td>8.2±2.8</td>
<td>4.0±3.2</td>
<td>3.9±3.1</td>
<td>0.1±0.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>PEG 1000 + Ferritin</td>
<td>4</td>
<td>20.6±6.0</td>
<td>13.4±3.2</td>
<td>18.2±2.6</td>
<td>10.5±1.4</td>
<td>7.3±0.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>PEG 1000 + Crocidolite</td>
<td>4</td>
<td>19.6±4.9</td>
<td>5.9±1.4</td>
<td>3.5±0.7</td>
<td>3.4±0.7</td>
<td>0.1±0.1</td>
<td>45.0±4.4</td>
</tr>
<tr>
<td>PEG 1000 + Crocidolite + desferrioxamine*</td>
<td>4</td>
<td>18.0±2.5</td>
<td>7.1±1.2</td>
<td>2.9±0.8</td>
<td>2.7±0.7</td>
<td>0.2±0.04</td>
<td>21.2±3.0</td>
</tr>
</tbody>
</table>

Values are given as means±sd. *: in a series of experiments on crocidolite exposed rats 250 mg desferrioxamine·kg⁻¹ body weight was added to the instillate. ± sig. diff. at p<0.001 from untreated control rats; † sig. diff. at p<0.01 from untreated control rats; ‡ sig. diff. at p<0.001 from rats instilled with only PEG 1000; § sig. diff. at p<0.01 from rats instilled with only PEG 1000; ¶ sig. diff. at p<0.001 from rats instilled with PEG 1000 and Crocidolite; PEG 1000: 10% + 1 ml·kg⁻¹ BW; Ferritin: 5 mg·kg⁻¹ BW; Crocidolite: 5 mg·kg⁻¹ BW; BW: body weight; n.d.: not detectable.
Desferal®, Ciba Geigy, Basel, Switzerland) to the instillate. In all groups urine was sampled over 24 h whereafter the rats were sacrificed and a bronchoalveolar lavage was performed as described above.

**Analysis**

The protein concentration in the bronchoalveolar lavage fluid was determined by use of the Lowry method [14], modified to be performed on 96-well microtitre plates and with BSA (A-7638, Sigma Chemical Co.) as the standard.

Polyethylene glycols were extracted from 1.0 ml urine or lavage fluid by using Kieselguhr columns (Extrelut, Merck, Darmstadt, Germany). The eluate obtained with 6.0 ml chloroform was evaporated under air whereafter 1.0 ml methanol: water (40:60) was added. The tube content was mixed on a Vortex mixer and placed in an ultrasonic water bath for 5 min. The different sized PEG 1000 polymers in the extracted samples were then determined after separation by reversed-phase high performance liquid chromatography (HPLC) as described elsewhere [15].

**Statistics**

All values are given as means±s.d. Student’s t-test were used for the statistical evaluations and the p-value <0.05 considered significant.

**Results**

As presented in table 1, there were no differences in total cell numbers or cell populations in the bronchoalveolar lavage fluid between untreated control rats and rats intratracheally instilled with the PEG 1000 marker solution alone. Also, no differences were obtained in the total protein levels in the lavage fluid between these groups. Intratracheal instillation of ferritin together with PEG 1000 resulted in significantly higher total cell numbers (p<0.01), a neutrophilia (p<0.001), and a higher total protein level (p<0.001) in the lavage fluid. By contrast, instillation of crocidolite did not change the total cell numbers or the total protein levels, but there was an increased number of mitoses of the alveolar macrophages 24 h after exposure (table 1). Furthermore, the addition of the iron chelator, desferrioxamine, had no effects on these parameters.

After intravenous injection of PEG 1000, the 24 h urinary recoveries of the different sized PEGs increased slightly from approx. 60 to 70% with increasing molecular weights (fig. 1). The molecular weight profiles were similar for the 30 days old rats and the adult rats as well as the ratios between the recoveries of PEG 1162 and PEG 810 (PEG 1162/810: 1.31±0.04 for the 30 days old rats and 1.22±0.10 for the adult rats).

After intratracheal instillation of PEG 1000, the urinary recoveries decreased from 60% to about 30% with increasing PEG molecular weight (fig. 2). The decrease was especially obvious for PEGs in the low molecular weight range 722-854 Da. There were no significant differences in the urinary PEG recovery between the 30 day old rats and the adult rats (fig. 2), nor in the PEG 1162/810 recovery ratios (0.78±0.25 vs. 0.69±0.27). The amounts of PEG remaining 24 h after instillation in the bronchoalveolar lavage fluid were small (5-10%), with a tendency to an inverted molecular weight profile as compared to the urinary recovery profiles (data not shown).
Urinary recovery of the molecular weights 854-1206 Da as compared to the normal rats (fig. 4), and the molecular weight profile was altered showing almost no molecular weight selectivity. A significantly higher PEG 1162/810 recovery ratio was obtained (1.47±0.59, p<0.01 vs control rats). Addition of desferrioxamine to the crocidolite instillate reduced the urinary PEG 1000 recovery levels and lowered the PEG 1162/810 recovery ratio to 0.97±0.47 (p<0.05 vs. control rats).

Discussion

This investigation shows that after an intratracheal instillation in rats, polyethylene glycols in the MW-range 722-1294 Da (PEG 1000) pass through the lower respiratory tract into the blood circulation and are recovered in the urine to an extent that depends on the molecular weight of the specific PEG polymer. The overall passage of 30-60% of PEG 1000 via the lower respiratory tract appears high, as compared with the passage across the gastrointestinal tract, which is less than 20-40% in rats (Folkesson et al., unpublished data). Intratracheal instillation was chosen as the method for delivery because it is easy to perform and offers an accurate and controlled deposition in the lungs [16]. No signs of subsequent lung inflammation due to the instillation procedure and/or the PEG:s used were found, since no increases were obtained in the inflammatory parameters measured in the bronchoalveolar lavage fluid.

The amounts of PEG eliminated from the blood circulation after intravenous administration were between 60-70% over a 24 h period. This is in agreement with a recent study, where approximately 65% of intravenously administered PEG 1000 was recovered in the urine within 6 h [17]. Moreover, the data obtained indicate that there were no differences in the elimination capacity from the blood into urine between the 30 days old and the adult rats. Thus, there were no apparent age differences in the ability of the kidney to sort out different-sized PEG polymers. If the obtained elimination efficiency of the PEG:s were taken into account, the urinary recovery values became almost 100% for the smallest PEG:s and up to 60% for the larger ones.

Our results show a molecular weight-dependent passage of the PEG:s with molecular weights less than about 850 Da, while the passage of the larger PEG:s appeared less molecular weight-dependent. In a study where different PEG mixtures were used to assess gastrointestinal and nasal permeability, little molecular weight dependence within the PEG 1000 and 2000 range was found, while a more distinct dependence was found in the PEG 600 range [17]. It was also shown that the nasal absorption of PEG 1000 and 2000 was 50-100% higher than the gastrointestinal absorption, while no such differences were found in the lower molecular weight range with PEG 600. These results taken together suggest a shift in the molecular weight selectivity of the pulmonary epithelium around 850 Da.
The lung passage of PEG:s in the MW-range 678-1250 Da was found to be similar in the younger and the adult rats. In an earlier study we showed that dDAVP, with a molecular weight of 1067 Da, passed through the lungs to a higher degree in 30 days old rats [3]. Schanker and Hemberger [2] has shown that low molecular weight compounds, ranging from 100-1,400 Da, pass via the lungs about twice as rapidly in 6 days old rats as in adult rats, while molecules in the 5,000-20,000 Da range pass at similar rates. The lipophility of the compounds is of importance for the lung passages according to Hemberger and Schanker [18]. They have shown that lipid-soluble compounds generally pass over the lung epithelium to a higher degree than lipid-insoluble compounds; lipid-insoluble compounds pass to a higher degree in six days old rats compared to adult rats, while lipid-soluble compounds are absorbed to a similar extent in both age groups. This might explain our different results obtained from studying dDAVP and PEG 1000 since PEG 1000 is more lipid-soluble than dDAVP.

We previously found that intratracheal instillation of ferritin markedly affected the lungs resulting in increases in cell numbers and total protein levels in the lung lavage fluid and causing an increased lung passage of proteins and the peptide dDAVP [9]. This effect appeared to be due to the ferritin iron content, since addition of the iron chelator, desferrioxamine, decreased the lung passage of the markers towards control levels. In the present study the total cell numbers were markedly increased due to a neutrophil invasion into the lungs after ferritin instillation, a result in accordance with our earlier study [9]. Furthermore, total protein levels in the lavage fluid were also increased, indicating the presence of alveolar oedema and a leaky blood-lung barrier. The urinary recovery of PEG polymers with a molecular weight <1030 Da was unaffected by the ferritin instillation, while the passage of the larger PEG:s was significantly increased. These results imply that the ability of the airway epithelium to discriminate between different-sized molecules and to function as a barrier against the penetration of larger PEG molecules, is impaired during inflammation. It is possible that larger PEG:s pass through the lungs mainly via a route that is affected by the inflammatory changes, e.g. paracellulary through the tight junctions.

The acute changes in the lungs following intratracheal instillation of crocidolite asbestos fibres caused the urinary PEG recovery to be significantly increased with cell numbers in the lavage fluid were unchanged both directly after exposure and 2 days later. The increased PEG 1000 passage might occur as a result from damage by the fibres themselves, as it has been shown that the alveolar epithelial cells might be injured by transepithelial migration into the interstitium of fibres [21, 22], thereby making physical "holes" in the epithelium. The importance of iron in the asbestos fibres was shown by the addition of desferrioxamine, which reduced the PEG passage. Earlier studies have shown that desferrioxamine binds to asbestos fibres both in vitro and in vivo and therefore might protect the lung tissue against oxidative injury [23]. Iron may participate in the iron-catalysed Haber-Weiss reaction leading to deleterious radical formation. An extended radical formation in the lungs will result in an increased oxidative damage to the tissue and might finally lead to increased marker lung passage. Thus, the findings are consistent with the recent proposal that iron-catalysed reactions may be responsible for the biochemical effects of asbestos fibres, regarding toxicity and cancer [11].

This investigation shows that PEG 1000 polymers pass over the respiratory tract of adult and 30 days old rats in large amounts and dependent on the molecular weight. The PEG passage was increased after intratracheal instillation of ferritin or crocidolite, indicating that the ability of the epithelial lining of the respiratory tract to discriminate between the different-sized PEG:s and to function as a barrier was impaired. It is suggested that the increased PEG passage under these conditions was due to local tissue damage induced by ferritin and crocidolite fibres, respectively, and that iron may play an important role in the toxic action of both these agents.

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References


