The distribution of myeloperoxidase, eosinophil cationic protein, albumin and urea in sequential bronchoalveolar lavage

B. Schmekel*, P. Venge**

The distribution of myeloperoxidase (MPO) and eosinophil cationic protein (ECP), secreted from activated neutrophils and eosinophils, was estimated in bronchoalveolar lavage fluid in a sequential lavage study performed on 12 healthy subjects. Four 50 ml aliquots were sequentially injected into the right middle lobe and immediately aspirated. Recent studies, using radiological methods, have revealed proximal airway distribution of the first infused lavage aliquot, and more peripheral distribution of the following ones. We found significantly higher concentrations of MPO (p<0.001) and ECP (p<0.001) in the first aspirated aliquots as compared to the following three. These findings are compatible with the concept that these substances are, to a substantial part, distributed to the surface of the proximal airways. In contrast, the sequential recovery of albumin and urea showed a homogeneous recovery pattern. The findings were compatible with those of a small series of sixteen 10 ml lavage aliquots, sequentially infused and aspirated, also indicating a continuous diffusion of these small molecules through the lung membranes into the lavage fluid during the lavage process. We conclude that the difference in recovery pattern and distribution on the bronchial surface makes albumin and urea unsuitable as denominators in ratios to MPO and ECP, for the estimation of quantitative local concentration in epithelial lining fluid.


Material and methods

Twelve healthy volunteers, with mean age 37.5 yrs (range 22-58 yrs) were subjected to bronchoalveolar lavage. Two of the subjects were female, five were tobacco smokers (mean 39.4 pack yrs, range 12.5-66 pack yrs). Routine spirometry was performed with a water sealed spirometer, and vital capacity (VC) and forced expiratory flow in one second (FEV1) were within 20% deviation from expected values [6]. Bronchoalveolar lavage was carried out in a standardized way. The subjects received an intramuscular injection of morphine-scopolamine and lidocaine (Xylocaine®, Astra, Sweden) was applied topically to the nasal and pharyngeal mucosa during the hour prior to the bronchoscopy. Lidocaine was also infused via the bronchoscope in order to achieve appropriate anaesthesia of the airway mucosa. A total of 305±34.2 mg (mean±sd) lidocaine was locally applied on the
airway mucosal membranes in connection with the bronchoscopy. The fiberoptic bronchoscope (Olympus BF 1 T10) was introduced via the nasal pathway to the larynx and trachea. With the bronchoscope in a wedge position in an anterior subsegment of the middle lobe, four aliquots of 50 ml warm sterile saline were sequentially infused and gently aspirated immediately after each infusion. The mean time used for each aliquot, from the start of infusion to the end of aspiration, was 1.7±0.3 min (mean±sd). The aspirated aliquots were collected in separate siliconized containers kept on ice.

In a second series of lavage, three of the male subjects were relavaged, at least six months after the initial one. Sixteen aliquots of 10 ml saline was sequentially injected and immediately aspirated. Fifteen to 20 seconds passed from the start of injection to the end of each aspiration. The aspirated lavage fluid was centrifuged at +4°C at 200 × g for ten minutes, and kept frozen at -70°C. Total cell counts and viability tests (Trypan blue inclusion) were performed in Bürker chambers. Differential counts were performed on cytopsin preparations (Shandon, Cytospin 2, England; at 500 rpm for 3 min). The preparations were stained with May-Grünwald-Giemsa and a total of 800 cells were counted and differentiated.

The concentration of myeloperoxidase (MPO), eosinophil cationic protein (ECP), albumin and urea were measured by previously described methods [7-10]. The total amounts of the substances were calculated by multiplying the concentration and the aspirated volume of the respective aliquot.

Statistical methods

Wilcoxon's rank sum test was used for comparison of values in groups.

Results

The volumes of sequentially aspirated aliquots tended to increase within the series, and the volume of the first aspirated aliquot was significantly lower than the second, third and fourth aliquots (p<0.01, p<0.001, p<0.001, respectively) (table 1).

The variability of BAL concentrations of MPO and ECP was large, and there was no statistical difference between the concentrations of the substances obtained from smokers and nonsmokers in the present series. The concentrations of MPO and ECP in the first aspirated aliquot were significantly higher than in the following aliquots (p<0.001 resp p<0.001), while the concentrations of albumin and urea remained unaltered throughout the series (fig. 1).

The aspirated total amounts of the four substances also varied greatly between the samples (table 2). The total amount of MPO recovered in the first aliquot was however significantly higher than in the second, third and fourth ones (p<0.01, p<0.01 and p<0.01, respectively). The total amounts of ECP also tended to decrease within the series, but the difference between total amounts recovered in the first and the following two aliquots did not reach statistical significance. No such decrements were observed in the total amounts of albumin or urea.

The accumulated total amounts aspirated in each of the four aliquots, were calculated for MPO, ECP, albumin and urea (fig. 2). Around 55% of the totally accumulated amounts of MPO was aspirated in the first aliquot, while around 30% of ECP and less than 20% of albumin or urea was collected in the first aspirated aliquot.

The recovery of alveolar macrophages was significantly higher in the second, third and fourth aliquot aspirated from smokers as compared to nonsmokers (p<0.05, p<0.01 and p<0.01, respectively, data not shown). The variability of neutrophil cell numbers was large in lavage aspirated from smokers, but there was no significant difference between the number of neutrophils or eosinophils in the aspirated fluids obtained from smokers and nonsmokers. Neither did the total counts of lymphocytes, nor the volumes of aspirated fluid differ between smokers and nonsmokers. Data on the cell recoveries from all of the twelve healthy subjects are shown in figure 3. There were no significant correlations between concentrations of neutrophils or eosinophils and the corresponding secretory products MPO or ECP. Furthermore, the neutrophil-MPO ratio, as well as the eosinophil-ECP ratio, varied greatly and tended to be lower in the first aliquot as compared to the following ones (data not shown).

<table>
<thead>
<tr>
<th>Table 1. - Pulmonary lavage volume in 12 healthy volunteers</th>
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<tr>
<td></td>
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<tr>
<td>Instilled volume ml</td>
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<td>Fluid recovered ml</td>
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Mean (sd) are given and significant difference between the first and the following aspirated aliquots are given (**: p<0.01; ***: p<0.001).
INFLAMMATORY CELL PRODUCTS IN LAVAGE FLUID

Fig. 1. – The concentration of (a) myeloperoxidase (MPO), (b) eosinophil cationic protein (ECP), (c) albumin, and (d) urea in 50 ml lavage aliquots, sequentially aspirated by bronchoalveolar lavage (BAL) from 12 healthy subjects. Mean values are given, standard error of means are indicated by vertical bars. Significant differences between concentrations measured in the first and the second aliquots are indicated (**: p<0.001).

Table 2. – Total amounts of myeloperoxidase, eosinophil cationic protein, albumin and urea in four 50 ml sequential lavage aliquots infused and aspirated from 12 healthy volunteers.

<table>
<thead>
<tr>
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<th>Aliquot 1</th>
<th>Aliquot 2</th>
<th>Aliquot 3</th>
<th>Aliquot 4</th>
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<tbody>
<tr>
<td>MPO μg</td>
<td>750 (92–6136)</td>
<td>209 (59–2178)**</td>
<td>255 (81–1369)**</td>
<td>126 (55–600)**</td>
</tr>
<tr>
<td>ECP μg</td>
<td>191 (115–468)</td>
<td>176 (83–339)</td>
<td>120 (49–367)</td>
<td>122 (80–205)*</td>
</tr>
<tr>
<td>Albumin mg</td>
<td>1.2 (0.7–1.8)</td>
<td>1.8 (1.3–3.0)</td>
<td>2.5 (1.4–3.1)</td>
<td>1.9 (1.4–3.0)</td>
</tr>
<tr>
<td>Urea μmol</td>
<td>0.5 (0.2–2.1)</td>
<td>1.4 (0.4–0.3)</td>
<td>1.5 (0.8–4.1)</td>
<td>1.9 (0.6–2.7)</td>
</tr>
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</table>

Median values and ranges are given. The statistically significant difference between values as compared with those of the first aspirated aliquot, is indicated (**: p<0.01, *: p<0.05). MPO: myeloperoxidase; ECP: eosinophil cationic protein.

Three of the subjects also underwent a 16 sequential lavage of 10 ml infused in each sequence. The volumes of the aspirated fluid, varied between 0.5 and 8 ml (mean 4.9 ml). The concentrations of MPO in the first of the aspirated aliquots (285, 462, 91 μg.l⁻¹, respectively) decremented throughout the series, to reach low levels (0.8, 1.3, 1.8 μg.l⁻¹, respectively) in the last aliquots. Also the concentrations of ECP decreased within the series (33, 15, 14 μg.l⁻¹, and 1.3, 2, 2 μg.l⁻¹, respectively). The concentrations of albumin were low in the first aliquot (0.005, 0.0005 and 0.10 g.l⁻¹) and varied greatly within the series, but showed a slight tendency to decrease throughout the series. The concentrations of urea, on the other hand, were unaltered throughout the series of 16 aspirated aliquots.
Fig. 2. - Percentage of the accumulated total number of the substance recovered in each of four sequential lavage aliquots aspirated from 12 healthy subjects. (a) myeloperoxidase (MPO), (b) eosinophil cationic protein (ECP), (c) albumin, and (d) urea. Mean values are given and SEM is indicated by vertical bars.

Fig. 3. - Total and differential counts of cells aspirated in four sequential bronchoalveolar lavages obtained from seven healthy nonsmokers and five healthy smokers. Mean values and standard deviations are given. ALL: total cells counts; AM: alveolar macrophages; LY: lymphocytes; NEU: neutrophils; EOS: eosinophils. Statistically significant difference between values as compared with those of the first aspirated aliquot is indicated (*: p<0.001; **: p<0.01; ***: p<0.05). ▲: aliquot 1; ▼: aliquot 2; ▼▼: aliquot 3; ▼▼▼: aliquot 4.
Fig. 4. – The percentage of the total accumulated amount of the substance recovered in each of 16 aliquots aspirated from three healthy subjects (a) myeloperoxidase (MPO), (b) eosinophil cationic protein (ECP), (c) albumin, (d) urea.

The total amounts of the substances were calculated and the proportions of the accumulated total amounts recovered in each of the serial aliquots are illustrated in figure 4. The accumulated total amounts of MPO reached a plateau after 5–6 aspirated aliquots, while ECP tended to reach a plateau after 5–10 aspirated aliquots. Due to the variability in the measured concentrations of albumin, the percentages of the accumulated total amounts varied greatly, but still had a tendency to level off after around 10 aspirated aliquots. The accumulated total amounts of urea, on the other hand, never reached a plateau but continued to increase within the series of aspirated aliquots.

Discussion

This investigation was carried out to assess the sequential recovery of MPO, ECP, albumin and urea in BAL fluid. The first aliquot infused in a series of sequential lavage aliquots, was previously reported to be distributed to the proximal bronchi, while the following ones washed airway mucosa in more distal airways [1]. We found that both the concentration and the total amount of MPO in lavage was highest in the first of a series of four sequentially infused and aspirated 50 ml aliquots. Thus, a substantial part of the aspirated MPO was located in the proximal bronchi. These findings are consistent with the distribution of neutrophils mainly to the proximal parts of the airways. Similarly, ECP also tended to be distributed to the proximal airways.

In our 16 sequential series, the small volume of saline that was injected, could not reach far from the tip of the bronchoscope in the airway lumen, until it was aspirated. Consequently, a small area of the proximal bronchial mucosa was lavaged by these small infusion volumes, and we postulated that the lavaged mucosal area was constant throughout the 16 sequential series. The plateau in the accumulated total amounts of MPO or ECP that were aspirated in the first five or ten aliquots, respectively, indicates that no more of the respective substance could be aspirated. This in turn suggests that MPO or ECP was washed away from the surface and did not enter this area during the lavage procedure.
The previously described abundance of neutrophils recovered in the first aspirated aliquots, *i.e.* the proximal airways [11], was confirmed in the present series. The co-variation of neutrophils or eosinophils and their secretory products MPO or ECP, suggests that the bulk of these soluble substances are secreted by the resident cells. Passive diffusion of MPO or ECP through the lung membranes, therefore appears not to add to the recovered amounts of these substances. Furthermore, the kinetic recovery of soluble substances and cells may not be governed by the same rules and, furthermore, preceding cell-activating events may alter the local concentration of soluble mediators such as MPO or ECP, due to enhancement of secretion or elimination by phagocytosis. Such imbalance in secretion or elimination of mediators may have influenced the large variability in neutrophil-MPO or eosinophil-ECP ratios in the present material.

The unaltered levels of the concentrations or total amounts of urea, both in the series of small 10 ml, and larger 50 ml sequential lavages, suggest a constant flow of this molecule into the lavage fluid during the lavage procedure. The transfer of urea through membranes during the lavage procedure has been reported previously [12] and it was suggested that a contact time of the lavage with the bronchial mucosa, over one minute would interfere with the recovery of urea [13]. The contact times, as defined by the time from the start of infusion of saline to the end of aspiration, were usually shorter than two minutes in the 50 ml sequential lavages, and in the 16 sequential lavages a contact time shorter than 20 s was achieved for every separate aliquot. However, since not all of the fluid was aspirated directly after the infusion, especially in the first aspirate, parts of that aliquot could also have been harvested in the following samples. Therefore, the following aspirates may have stayed in contact with the lung membranes during a longer period. Consequently, the successive rise in the accumulated total amounts of urea, could be the result of an uncontrolled long contact time, even in the 16 sequential lavages.

There have been no reports on the influence of contact time of lavage fluid and the recovery of albumin. The successive increases of the total amounts of albumin were similar to those of the urea amounts in the 50 ml aliquots, and there were no significant differences between the proportions of accumulated amounts of albumin and urea in the separate aliquots in the 4 sequential lavages. The concentrations of albumin in the 16 sequential lavages were so low that the plateau in accumulated total amounts that was reached after 10 aliquots may well depend on the uncertainty in measurements of the low concentrations of albumin. The finding that urea is continuously transferred into the bronchial lumen during the lavage procedure, may therefore also be valid for albumin, at least in a series of 50 ml aliquots. In accordance with previous reports [11], it therefore seems inappropriate to use urea or albumin as denominators in protein ratios to estimate the local concentrations in epithelial lining fluid.

The finding of lower recovery volumes in subjects with obstructive airway disease [14] may be a result of decreased amounts of supportive structures in the peripheral parts of the airway tree, leading to increased flaccidity of the airway walls and easily induced closure of these airways. This, in turn, implies that the injected fluid will have no access to the most peripherally located parts of the airway mucosa. Higher concentrations of MPO and ECP would then be measured in the fluids aspirated from such subjects, due to the absence of dilution by fluid with lower concentration of the substance, originating from more peripheral parts of the airway tree. Consequently, any therapeutic intervention leading to bronchodilatation, and as a result of that a more peripherally located lavage area, may show decrements in lavage concentrations of MPO and ECP, independently of the inflammatory cell response.

In conclusion, our findings suggest that MPO and ECP to a substantial extent are located on the mucosa of the proximal airways. Neither MPO nor ECP appears to diffuse through the mucosal membrane during the lavage procedure. The observation that urea and albumin appear to pass through membranes during a standard lavage procedure argues against the use of those two proteins as denominators in ratios of lavage fluid to estimate the local protein concentrations of other substances.

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**References**

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La distribution de la myéloperoxidase (MPO), de la protéine éosinophile cationique (ECP), de l’albumine et de l’urée, lors de lavages bronchoalvéolaires séquentiels. B. Schmekel, P. Venge.

RÉSUMÉ: La distribution de la myéloperoxidase (MPO) et de la protéine éosinophile cationique (ECP) sécrétées par des neutrophiles activés et des éosinophiles, a été estimée dans le liquide de lavage bronchoalvéolaire dans une étude de lavages séquentiels conduite chez 12 sujets bien portants. Quatre aliquots de 50 ml ont été injectés séquentiellement dans le lobe moyen droit et réaspirés immédiatement. Des études récentes, reposant sur des méthodes radiologiques, ont révélé une distribution du premier aliquot de lavage infusé au niveau des voies aériennes proximales, et une distribution plus périphérique des aliquots suivants. Nous avons trouvé des concentrations significativement plus élevées de MPO (p<0.001) et de ECP (p<0.001) dans les premiers aliquots aspirés par comparaison aux trois suivants. Ces observations sont compatibles avec la conception selon laquelle ces substances sont, pour une part substantielle, distribuées à la surface des voies aériennes proximales. Au contraire, le recueil séquentiel d’albumine et d’urée a montré un type très homogène de récupération. Ces observations sont compatibles avec celles d’une petite série de 16 aliquots de lavage de 10 ml, qui ont été injectés et réaspirés de manière séquentielle, et qui indiquaient également une diffusion continue de ces petites molécules au travers des membranes pulmonaires dans le liquide de lavage pendant le processus du lavage. Nous concluons que la différence dans le type de récupération et de distribution sur les surfaces bronchiques fait de l’albumine et de l’urée des dénominateurs non adéquats dans les ratios avec MPO et ECP, pour l’estimation de la concentration locale quantitative dans les liquides de recouvrement épithelial.