Endotoxin Removal by End-Line Filters

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Received 3 April 1989/Accepted 16 August 1989

Four commonly used end-line filters, one with a charge-modified hydrophilic nylon filter (ELD96; Pall Biomedical Ltd., Portsmouth, United Kingdom), one with an unmodified nylon filter (FAE020; Pall Biomedical), and two with hydrophilic cellulose ester filters (Ivex-HP, Millipore Corp., Bedford, Mass.; Sterifix, Braun-Gelman, Brussels, Belgium), were evaluated for their endotoxin-removing capacity in saline and 5% glucose. Natural endotoxins derived from Escherichia coli 8739 and the lipopolysaccharide mutant Pseudomonas aeruginosa PA220-R2 and a purified E. coli serotype O111:B4 lipopolysaccharide preparation were used to challenge the four end-line filters. No endotoxin-removing capacity was observed for the Ivex-HP and Sterifix filters. Both the FAE020 and the ELD96 end-line filters showed excellent endotoxin-eliminating capacities in 5% glucose. Increasing the NaCl concentration in 5% glucose, however, greatly reduced the endotoxin-removing efficiency of especially the ELD96 filter for the purified E. coli lipopolysaccharide preparation. Obviously, both the nature of the endotoxin and the ionic strength of the solution had a major influence on the endotoxin end-line filtering efficiency.

End-line filters are used at the end of an intravenous (i.v.) administration set to eliminate particulate matter, microorganisms, and air from the infusion fluid (4, 9, 15). Microbiological contamination of the infusion system is, together with wound, urinary tract, and pulmonary infections, a major cause of sepsis with postoperative patients (11). Most microorganisms that survive and proliferate in i.v. solutions are gram-negative bacteria (2, 7). Although it is generally accepted that certain antibiotics have the capacity to induce the release of endotoxins from gram-negative bacteria (14), spontaneous release of endotoxins as lipopolysaccharide (LPS)-protein vesicles in the surrounding aqueous medium has been reported (3).

A variety of methods are available for removing endotoxins from aqueous solutions (9, 10, 15). In view of the clinical implications of endotoxin removal from contaminated i.v. fluids, a comparison was made between the endotoxin-eliminating capacity of four filters; both natural and purified endotoxin preparations were used to challenge the end-line filters.

MATERIALS AND METHODS

Bacterial strain and LPS preparation. Escherichia coli 8739 was obtained from the American Type Culture Collection (Rockville, Md.). Pseudomonas aeruginosa 220-R2 was an LPS mutant strain, kindly provided by S. Cryz, Swiss Serum and Vaccine Institute, Bern, Switzerland. After 7 h of growth on tryptic soy agar (Oxoid Ltd., Basingstoke, United Kingdom) at 37°C, a nutrient agar plate (Oxoid) was massively inoculated with E. coli or P. aeruginosa. After growth for 17.5 h, the bacteria were scraped off the agar plates, collected by centrifugation, and suspended in 5.0 ml of water for injection (Travenol Inc., Lessines, Belgium). The suspension was centrifuged, and the supernatant was filtered through a membrane filter (0.45-μm pore size; Millipore Inc., Brussels, Belgium). This stock solution of natural endotoxins was stored at 4°C for a maximum of 1 week. The purified E. coli serotype O111:B4 endotoxin preparation was obtained from Sigma Chemical Co. (St. Louis, Mo.).

Endotoxin testing. Endotoxin activity was measured by making use of a chromogenic Limulus amoebocyte lysate assay (Kabi Diagnostica, Stockholm, Sweden) as described elsewhere (17). The sensitivity of the assay was equal to 5 pg of the purified E. coli serotype O111:B4 endotoxin (Kabi Diagnostica) per ml (1 EU = 80 pg of the reference E. coli O111:B4 endotoxin).

i.v. filter evaluation. Four commonly used end-line filters (4) with a charge-modified hydrophilic nylon filter and a small hydrophobic PTFE air-filter (ELD96; Pall Biomedical Ltd., Portsmouth, United Kingdom), an unmodified nylon filter and a large hydrophobic PTFE membrane filter (FAE020; Pall Biomedical), or hydrophilic cellulose ester filters (Ivex-HP, Millipore Corp., Bedford, Mass.; Sterifix, Braun-Gelman, Brussels, Belgium) were submitted to the challenge experiments.

i.v. administration simulation. To be able to connect a polyvinyl chloride or polybutadiene i.v. administration set for delivery of the endotoxin solution, borosilicate beakers were modified. At the end of the administration set an end-line filter was attached. To determine the endotoxin levels in both the solutions in the borosilicate beaker and in the filtered solutions, 4-ml samples were taken after 0, 5, 10, 15, 30, 45, and 60 min. The flow rate was approximately 110 ml/h and was determined by measuring the total amount of fluid passed through the filter. To evaluate the influence of the NaCl concentration on the endotoxin-capturing properties of the ELD96 and FAE020 filters, challenge experiments were performed with the purified E. coli serotype O111:B4 endotoxin in 5% glucose containing different NaCl concentrations (0, 0.10, 0.225, 0.445, and 0.9%).

ECC determination. Expressed as picograms of the reference E. coli serotype O111:B4 endotoxin (Kabi Diagnostica), the endotoxin-capturing capacity (ECC) could be calculated from the plot of the percentage of endotoxins captured by the filter at a given endotoxin load versus the effluent time. The ECC represented the amount of endotoxin adsorbed on the membrane surface of one end-line filter, before endotoxins could be detected in the effluents.

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TABLE 1. ECCs* of the end-line filters

<table>
<thead>
<tr>
<th>Endotoxin</th>
<th>Challenge solution</th>
<th>ECC (pg of endotoxin)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>FAE020</td>
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<tr>
<td><em>E. coli</em> serotype O111:B4, purified</td>
<td>5% Glucose</td>
<td>2.84 x 10^7 ± 1.45 x 10^7</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 8739, natural</td>
<td>5% Glucose</td>
<td>1.89 x 10^7 ± 1.45 x 10^7</td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl</td>
<td>1.49 x 10^6 ± 0.78 x 10^6</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> 220-R2, natural</td>
<td>5% Glucose</td>
<td>1.42 x 10^1 ± 1.02 x 10^3</td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl</td>
<td>1.82 x 10^2 ± 0.49 x 10^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.18 x 10^5 ± 0.23 x 10^5</td>
</tr>
</tbody>
</table>

*The maximal amount of endotoxin a filter can capture before endotoxins are detectable in the effluent. Ivex-HP and Sterifix filters were unreliable for endotoxin elimination.

RESULTS

Challenge experiments in 5% glucose. Even at the lowest endotoxin load of 1 ng of endotoxins per ml in the challenging solution, no reliable endotoxin elimination by the cellulose ester end-line filters (Ivex-HP and Sterifix) could be detected. For the ELR96 and FAE020 filters excellent retentive capacities were observed, especially for the purified *E. coli* preparation (Table 1).

Challenge experiments in 0.9% NaCl. The results obtained for the end-line filters with a cellulose ester hydrophilic membrane, Ivex-HP and Sterifix, were consistent with those found in 5% glucose. In contrast to the unchanged ECCs for the natural *P. aeruginosa* 220-R2 LPS, no reliable endotoxin elimination could be ascribed to the ELR96 filter in a normal saline solution for the purified *E. coli* endotoxin preparation (Table 1). However, although lower ECCs were measured than in glucose, the FAE020 filter retained a significant endotoxin-removing efficiency, especially for the purified *E. coli* and natural *P. aeruginosa* LPSs (Table 1).

Influence of the NaCl concentration on the ECCs in glucose. With the purified *E. coli* O111:B4 LPS preparation, an increase in the NaCl concentration in 5% glucose resulted in a reduction of the ECCs or the endotoxin capture efficiency, especially for the ELR96 end-line filter (Fig. 1). The pH of the 5% glucose solution did not change significantly (n = 6) upon NaCl addition (pH 4.58 ± 0.01).

DISCUSSION

One of the most common effects of i.v. injected endotoxins is the development of fever (6). Fever, however, is probably one of the least dangerous of the numerous effects of the bacterial LPS. Next to pulmonary (8) and cardiodepressor effects (6), major influences on the immunologic system, such as the activation of the B lymphocytes and mononuclear cells, and the stimulation of the release of interleukin-1, interferon, prostaglandines, leukotrienes, and tumor-necrotizing factors have been ascribed to i.v. administered endotoxins (11). Since gram-negative bacterial contaminations still occur within the hospital setting itself (5, 13), the use of end-line filters with an endotoxin-eliminating capacity during i.v. therapy is recommended (5).

In the present study, an evaluation is made of the endotoxin-filtering efficiency of four end-line filters. To avoid secondary bacterial adsorption effects, no use was made of whole bacterial cells in the endotoxin challenge experiments, but three different LPS preparations, one purified *E. coli* serotype O111:B4 LPS and two natural LPS preparations from *E. coli* 8739 and the LPS mutant *P. aeruginosa* 220-R2 were utilized.

No reliable endotoxin-capturing properties could be determined for the two end-line filters with hydrophilic cellulose membranes (Ivex-HP and Sterifix). Since both filters have an unmodified hydrophilic membrane and a small hydrophobic surface, such results were not unexpected. With the FAE020 filter, which was not expected to possess endotoxin-capturing properties, astonishing high ECCs were obtained. According to Pearson (12), polyamide (nylon) membranes exhibit overall net positive charges in aqueous solutions with a pH below 9.0. Since the FAE020 has such a hydrophilic nylon membrane (Ultipor), the endotoxin retention properties could be mediated by electrostatic interaction forces. However, this is in contrast with the data provided by the manufacturer, attributing only a positive zeta potential of the Ultipor N 00 membrane (FAE020 filter) below pH 6.0. It should be mentioned, however, that streaming potential measurements would be a better indication than the zeta potential determinations for the electrokinetic properties of the filter membrane surface. Whether the large hydrophobic membrane, introduced to allow air to be vented out of the infusion fluid, or the different filter housings are of significant importance in this endotoxin-filtering process remains un-

FIG. 1. Influence of the NaCl concentration on the percent endotoxin capture (± standard deviation; n = 3) of the FAE020 (○, □, △, ○) and ELR96 (■, ■, ▲, ■) filters at a load of 3 x 10^7 pg of the reference *E. coli* serotype O111:B4 endotoxin per ml in 5% glucose. NaCl concentrations: ○ and ●, 0%; □ and ■, 0.1%; △ and ▲, 0.225%; ◆ and ●, 0.9%.
clear. Several authors have suggested the existence of a hydrophobic adsorption mechanism in the removal of endotoxins from aqueous solutions by hydrophobic polymers such as polypropylene, polyethylene, polyvinylidene fluoride, and polytetrafluoroethylene (9, 10, 12). However, taking into account the physical aggregation state of endotoxins (1, 16), an interaction between the hydrophobic lipid A part of the LPS molecule and the polymer surface is thermodynamically unlikely.

With purified E. coli serotype O111:B4 LPS, a decrease in endotoxin-filtering efficiency of the ELD96 and FAE020 filters occurred with increasing NaCl concentrations in 5% glucose (Fig. 1). This could be due to a gradual masking of both the negative groups of the endotoxins and the positive groups of the hydrophilic filter membrane by increasing NaCl concentrations (9, 10). This effect was not observed for the natural P. aeruginosa 220-R2 LPS. In agreement with Meltzer (10), in comparing the ECCs of the filters in saline and in 0.9% NaCl in 5% glucose (Fig. 1, Table 1), only a slight influence of the osmolarity on the endotoxin-filtering efficiency may be expected.

Cost-benefit analysis is an important feature influencing the use and choice of an end-line filter (5). One way to reduce the cost is to increase the time of use of the device, and the ELD96 filter has been introduced in the hospital environment for that purpose. Due to its positively charged, modified hydrophilic membrane, important endotoxin-capturing properties are assigned to it. Although some authors found that charge-modified membranes completely lost their removal efficiency in the presence of an electrolyte (9), Baumgartner et al. observed a 100% endotoxin retention efficiency within a 96-h infusion period (2). Since only whole bacterial cells from one E. coli strain were used in the Baumgartner study, we consider that the effectiveness of the ELD96 filter for removing endotoxins has not been proven.

The endotoxin-filtering capacity of the ELD96 filter in 5% glucose was excellent, especially for the purified E. coli serotype O111:B4 and natural E. coli 8739 LPSs (Table 1). In contrast to the gradual appearance of endotoxins in the effluents of the FAE020 filter, a quite abrupt leakage of endotoxins through the ELD96 filter was observed. Obviously, once the available places on the Posidyone membrane surface were occupied, the ELD96 filter completely lost its endotoxin retention properties. The statement that the ELD96 filter has a 100% endotoxin-filtering efficiency should clearly be refined. In saline, even for the natural P. aeruginosa 220-R2 endotoxin, low ECCs were obtained.

In conclusion, the endotoxin elimination efficiencies of both the ELD96 and FAE020 filters were strongly dependent upon the nature of the contaminating endotoxin and the ionic strength of the solution. The other two end-line filters, Sterifix and the Ivex-HP, did not show any reliable endotoxin retention properties.

ACKNOWLEDGMENTS

We greatly acknowledge the valuable comments made by K. Van den Haesevelde and by G. Lowe and P. Ball of Pall Biomedical and the excellent technical assistance of R. Aerts.

LITERATURE CITED


