Evaluation of the in-line filters for the intravenous infusion of amphotericin B fluid

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SUMMARY
Objective: To evaluate the effects of four types of in-line filters on filtration rate, amphotericin B concentration and particulate matter.

Methods: Filtration rates of amphotericin B fluid through in-line filters under maximum gravity flow were examined. The concentrations of amphotericin B and the particulate matter in the non-filtered and filtered amphotericin B fluid at the flow rate of 500 ml/24 h were measured.

Results: Filtration through a 1-2 µm or 0-2 µm polyethersulphone (PES) filter under maximum gravity flow took less than 40 min. The 0-2 µm positively charged nylon 66 and 0-2 µm nylon 66 filters took 70 and 100 min, respectively, to filter 500 ml of amphotericin B fluid. The 0-2 µm positively charged nylon 66 filter and the 0-2 µm nylon 66 filter, but not the PES filter (1-2 and 0-2 µm), decreased the concentrations of amphotericin B in the filtered fluid by 100% within 1 h and by 66% within 24 h after the start of filtration, respectively. The particulate count in the non-filtered amphotericin B fluid was 27 ± 5 particles/ml, exceeding the limit defined by USP XXIII. The 1-2 µm and 0-2 µm PES filters significantly decreased particulate matter by 83 and 97%, respectively, just after filtration.

Conclusion: The present results indicate that the 0-2 µm PES filter is optimal for intravenous infusion of amphotericin B fluid to minimize the introduction of particulate matter, microbial contaminants and endotoxin into patients.

INTRODUCTION
Amphotericin B, an amphipathic polyene macrolide derived from Streptomyces nodosus, is widely used for many serious systemic fungal infections. Its application has increased during the past decade for the treatment of several fungal infections accompanied by organ and tissue transplantation, intensive chemotherapy and especially human immunodeficiency virus infection (1, 2). Amphotericin B is a water insoluble substance and is usually prepared as a micellar sodium deoxycholate (DOC) complex. The amphotericin B preparation, a commercially available DOC complex, is a colloidal dispersion with 82% of the particles less than 3 µm in size (3). This colloidal amphotericin B fluid causes toxic effects in patients, including renal dysfunction, anaphylaxis, chills, high fever, nausea, phlebitis, anorexia and venous irritation (4, 5), although the exact cause of these effects is not clearly defined. Recent lipid-associated or liposomal amphotericin B delivery formations have reduced these toxicities without decreasing antifungal activity (6, 7). However, these preparations are prohibitively expensive, especially in the developing world. Therefore, colloidal amphotericin B preparation is still needed for antifungal therapy.

The use of an in-line final filter during the administration of intravenous fluids can minimize the introduction of particulate matter, air and lipid emboli, microbial contaminants and endotoxins into patients (8). In-line filters reduce the incidence of infusion phlebitis, pulmonary artery granulomata and coronary vasoconstriction (9–11). However, in-line filters sometimes decrease the potency and flow rate of drugs, including amphotericin B (3, 12–14). The optimum pH range for preparing stable colloidal amphotericin B fluid is 6–7. At pH less than 6-0, the colloidal solution may become turbid. Addition of 5% USP dextrose is recommended for preparing intravenous amphotericin B infusion at pHs greater than 4-2, and supplemented fluid at pH 6-5 and 5-6 passed easily through 0-45 or 1-0 µm filter, respectively (12). Filtration with a 0-22 µm filter substantially decreased the concentrations of amphotericin B in the infusion, even at pH 6-5, several
Table 1. In-line final filters used

<table>
<thead>
<tr>
<th>Pore size (μm)</th>
<th>Type of filter</th>
<th>Filtration area (cm²)</th>
<th>Manufacturer</th>
<th>No. of filter kit&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lot no. of filter kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>polyethersulphone</td>
<td>12.84</td>
<td>Pall&lt;sup&gt;c&lt;/sup&gt;</td>
<td>FG-20AY&lt;sup&gt;d&lt;/sup&gt;</td>
<td>97E06</td>
</tr>
<tr>
<td>0.2</td>
<td>nylon 66&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.25</td>
<td>Pall&lt;sup&gt;c&lt;/sup&gt;</td>
<td>PDI&lt;sup&gt;f&lt;/sup&gt;</td>
<td>715602</td>
</tr>
<tr>
<td>0.2</td>
<td>Posidyne® positively charged nylon 66</td>
<td>11.25</td>
<td>Pall&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ELD96LYL&lt;sup&gt;g&lt;/sup&gt;</td>
<td>628807</td>
</tr>
<tr>
<td>1.2</td>
<td>polyethersulphone</td>
<td>10</td>
<td>Pall&lt;sup&gt;c&lt;/sup&gt;</td>
<td>FG-120BY-N&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96E09</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> In-line filter kits (b) are each composed of the filter (a) and the housing.
<sup>c</sup> Pall (USA, Web pages http://www.pall.com/)
<sup>d</sup> Manufacturer: Nipro (Osaka, Japan)
<sup>e</sup> Nylon 66 filter is available only in Japan
<sup>f,g</sup> Manufacturer: Pall Japan (Tokyo, Japan)

hours after filtration (12). Therefore, filters larger than 1 μm are recommended for the filtration of amphotericin B fluid (15). This recommendation needs to be reevaluated, as various new types of in-line filters have been recently developed. Based on the limit defined by United States Pharmacopoeia (USP XXIII), there should be less than 25 particles/ml of particulate matter larger than 10 μm in large volume injections (16). We have reported that particulate matter in non-filtered amphotericin B fluid was over this limit (40.8 ± 7.0 particles/ml, n = 3) when amphotericin B was reconstituted with 10 ml of sterile water and 5% dextrose (50 mg/500 ml) (17). The filtration of the reconstituted amphotericin B injection through a 0.2 μm polyethersulphone (PES) membrane filter before an addition of 5% dextrose significantly decreased particulate matter in the final solution by 64% without loss of amphotericin B (17). In the present study, we examined the effects of various in-line final filters on the flow rate of intravenous amphotericin B fluid and the concentration of amphotericin B in the filtered fluid using micellar electrokinetic capillary chromatography (MEKC). Particle size in amphotericin B fluid after filtration was also measured.

MATERIALS AND METHODS

Chemicals and in-line filters

The chemicals used were as follows: amphotericin B for infusion (50 mg Fungizone® injection, imported by Bristol-Myers Squibb, Tokyo, Japan, Lot No.; FZV1180), 5% dextrose for injection (500 ml; Otsuka, Lot No.; K8A77) and sterile water for injection (20 ml; Otsuka, Japan, Lot No.; K7G80). All remaining reagents were analytical grade and were purchased from Wako Pure Chemical Industries (Osaka, Japan). The in-line filters used are shown in Table 1. An intravenous administration set (Terufusion, Terumo Co. Ltd, Japan, Lot No.; 971020D1) was also used.

Preparation of standard solution

One vial of amphotericin B for infusion was reconstituted with 10 ml of sterile water and shaken for 5 min with a shaker (original amphotericin B solution: 50 mg/10 ml). One ml of this solution was diluted with 9 ml of 5% dextrose injection and then further dilutions (2.5, 5, 10, 20, 40, 60 and 100 μg/ml) were made with 5% dextrose injection for the calibration of the MEKC assay.

Preparation of samples

Flow rate of amphotericin B fluid during filtration

Based on the intended clinical application, either 4 or 10 ml of the original amphotericin B solution was added to 500 ml infusion vials of 5% dextrose to make 40 or 100 μg/ml amphotericin B concentrations. The fluid reservoir was hung 90 cm above the filter and intravenous fluid was dripped through in-line final
Evaluation of in-line filters

The volume of filtered fluid was measured every 10 min during filtration.

Concentrations of amphotericin B in the filtered fluid

Five bottles of intravenous amphotericin B fluids (40 µg/ml, 500 ml) were dripped through each in-line filter at an initial flow rate of 500 ml/24 h. One bottle was allowed to drip without filter as a control. One ml was sampled from the original bottle at the start of filtration (0 h). One ml was sampled directly from post-filtered fluid at 1, 2, 4, 8, 12, 18 and 24 h after the start of filtration. One ml was also sampled from the pooled fluid collected for 24 h after the start of filtration. Each sample (1 ml) was diluted with 5% dextrose for injection (1:100) and analysed by MEKC assays. Data were obtained from three sets of experiments. Two types of PES filters were tested further with amphotericin B fluid (100 µg/ml).

Particulate matter in amphotericin B fluid after filtration

Five ml of amphotericin B fluid was directly sampled at 0 and 24 h after the start of filtration through filters shown to be optimum by MEKC assays. Samples were degassed by aspirator for 4 min and the amount of particulate matter ranging from 2 to 100 µm in size in the media was measured.

MEKC assays of amphotericin B

Amphotericin B concentrations in pharmaceutical solutions were measured by the MEKC method, as described previously (17). MEKC assays, one type of capillary electrophoresis (CE), were performed on an HP3D system with a diode-array UV detector (Hewlett-Packard, Waldbronn, Germany). In all experiments, a constant voltage of 30 kV was applied, the temperature was set at 25°C and detection was made at 407 nm. A silica-fused extended light path capillary (64.5 cm × 75 µm i.d., Hewlett-Packard) was used for all MEKC separations (18). The composition of the electrophoresis running buffer was 10 mM phosphate buffer (pH 8.8) with 25 mM SDS. A sample was injected into the capillary with the vacuum system at 50 mmHg for 5 s. The mean of triplicate determinations was calculated.

Measurement of particulate matter

Particulate matter in pharmaceutical solutions was measured with a PMS counter as described previously (19). In brief, the PMS system consisted of an APSS-200 counter connected to a LiQuilaZ-E20 sensor with a laser diode (121 mW, 780 nm) and automatic bottle sampler, LS-200 (Particle Measuring Systems Inc., Boulder, CO, U.S.A.). The mean level of triplicate determinations was calculated.

RESULTS AND DISCUSSION

Changes in the flow rate of amphotericin B fluid

The pH of each intravenous amphotericin B fluid (40 and 100 µg/ml) was within that necessary for a stable colloidal solution (pH 6.7 and 7.0, respectively; n = 1). The volumes of intravenous amphotericin B fluid (40 µg/ml) filtered through various in-line filters under maximum gravity flow are shown in Fig. 1. Without an in-line filter, 500 ml amphotericin B fluid emptied completely within 20 min (control). Filtration through
Fig. 2. Concentrations of amphotericin B in intravenous fluid filtered with in-line filters at 0, 1, 2, 4, 8, 12, 18 and 24 h after filtration (○, \( n=3 \)). Change in flow rate of intravenous fluid initially set to 500 ml/24 h (●, \( n=1 \)). Five hundred ml of 5% dextrose containing 40 \( \mu \)g/ml of amphotericin B were used in each experiment. The concentrations are shown as the mean ± SD (\( n=3 \)). \( P<0.001 \) and \( P<0.0001 \) vs. non-filtered (repeated measures ANOVA).

A 1.2 \( \mu \)m or 0.2 \( \mu \)m PES filter took less than 40 min. The 0.2 \( \mu \)m positively charged nylon 66 and 0.2 \( \mu \)m nylon 66 filter took 70 and 100 min, respectively, to filter 500 ml of amphotericin B fluid (Fig. 1). Tests using amphotericin B fluid (100 \( \mu \)g/ml) were very similar.

In experiments where the flow rate was initially set to 500 ml/24 h, the 0.2 \( \mu \)m positively charged nylon 66 and the 0.2 \( \mu \)m nylon 66 filters decreased the flow rate by 20 and 50%, respectively, but the 1.2 \( \mu \)m or the 0.2 \( \mu \)m PES filters did not (each filter tested once, Fig. 2). Flow rate inhibition by the 0.2 \( \mu \)m positively charged nylon 66 filter was highest. These flow rates are close to those obtained with the maximum gravity flow experiments. This simple and time-saving experiment may be enough to estimate the effects of filters on flow rate of various intravenous fluids. The 1.2 \( \mu \)m and 0.2 \( \mu \)m PES filters are more suitable for use than the 0.2 \( \mu \)m positively charged nylon 66 and the 0.2 \( \mu \)m nylon 66 filters in terms of flow rate.
**Changes in amphotericin B concentrations of the filtered intravenous fluid**

The concentrations of amphotericin B in the intravenous fluid were measured after filtration with various filters (Fig. 2). The flow rate was set at 500 ml/24 h before the start of filtration. The 0.2 μm nylon 66 filter gradually decreased the concentration of amphotericin B to 47.1 ± 35.7% and 65.8 ± 34.7% at 18 and 24 h after the start of filtration, respectively. The 0.2 μm positively charged nylon 66 filter rapidly decreased amphotericin B concentrations to undetectable levels within 1 h; this event was accompanied by a slow filtration rate. The 1.2 μm and 0.2 μm PES filter did not influence amphotericin B concentrations in fluid filtered at each period during filtration or on the final pooled fluid filtered for 24 h [Fig. 2 (40 μg/ml) and Fig. 3 (100 μg/ml)]. Positively charged nylon 66 filters have amide linkages in the linear polymer and adsorb negatively charged substances such as pyrogens, bacteria, virus and colloidal materials from solutions (8). This type of filter is inappropriate for filtration of amphotericin B fluid because it adsorbs micellar amphotericin B/DOC complex and slowly becomes clogged. PES is less adsorptive and is particularly useful for filtration of protein products (8, 20). Filtration through PES membranes produces fewer conformational changes in proteins compared to nylon membranes (21). Although the bioactivity of amphotericin B fluid filtered through PES filter was not examined, the CE spectrum of amphotericin B was not affected. These findings show that 1.2 μm and 0.2 μm PES filters filter amphotericin B fluid without significant loss of amphotericin B.

**Particulate matter**

Figure 4 shows the numbers of particles larger than 10 μm in preparations of intravenous amphotericin B fluid filtered through 0.2 μm or 1.2 μm PES filters. Based on the limit defined by United States Pharmacopoeia (USP XXIII), there should be fewer than 25 particles/ml of particles larger than 10 μm in large volume injections. Particulate matter in the non-filtered intravenous amphotericin B fluid before filtration was 27 ± 5 particles/ml (Fig. 3). The 1.2 μm and 0.2 μm PES filter significantly decreased particulate matter by 83 and 97%, respectively, after filtration (Fig. 3). Dextrose is recommended for preparing the stable colloidal intravenous amphotericin B fluid at pH higher than 4.2. Five percent dextrose for injection was reported by the manufacturer to have a pH of around 4.8, but the pH of the 5% dextrose for injection used here was 4.6 (n = 1). These results indicate that 1.2 μm and 0.2 μm PES filters can be used to filter intravenous amphotericin B fluid prepared with 5% dextrose at pH greater than 4.2 and remove the larger particles from the fluid. In-line filter membranes no greater than 0.2 μm size are required to minimize the risk of infection in patients during the administration of intravenous fluids (9–11). Therefore, we recommend 0.2 μm...


REFERENCES


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