The use of lipid emulsions for the iv administration of a new water soluble polyene antibiotic, SPK-843

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Introduction

The therapeutic importance of the polyene antifungal agents arises from the fact that they constitute one of the few groups of drugs effective against fungal infections, mainly because of their capacity to interact with fungus cell membrane sterols. The polyenes are macrocyclic-structured substances characterized by the presence of a series of conjugated double bonds, essential for their antifungal activity, but at the same time contributing to their insolubility in water, typical of all the polyene antibiotics. This insolubility makes their administration by systemic routes very difficult unless complexes, colloidal dispersions or liposomal formulations are used.

To obtain products with strong antifungal activity and good water solubility, various partricin A derivatives were recently synthesized. One of these, N-dimethylaminoacetylpartricin A 2-dimethylaminoethylamide diascorbate, also called SPA-S-843 or SPK-843 (Figure), exhibits promising toxicity (LD50 69 mg/kg iv in mice and 9.3 mg/kg iv in rat) and microbiological activity (MIC 0.02 mg/L against Candida albicans and 0.3 mg/L against Aspergillus niger), together with high solubility in acidic or slightly acidic aqueous media, making it a candidate for clinical development in the near future.

During pre-clinical studies, mild adverse events (mostly phlebitis) connected with repeated intravenous administration of SPK-843 were reported. Therefore, a lipid emulsion (Intralipid), usually employed for parenteral nutrition, was proposed as the infusion vehicle for SPK-843, to replace the more usual glucose solution, with encouraging results. Studies in rats showed similar pharmacokinetic parameters for the substance formulated in the lipid emulsion or in glucose solution, but with higher tissue concentrations in the first case, particularly in the spleen, possibly because the lipid plays an active role in tissue distribution.

The same lipid emulsions were also recently proposed as the infusion vehicles for Fungizone [amphotericin B and sodium desoxycholate by Bristol-Myers Squibb (Sermoneta, Italy)], but some problems of physical incompatibility between amphotericin B and these vehicles indicated that more extensive studies of physical and chemical compatibility between SPK-843 and lipid emulsions and accurate investigations of venous toxicity were needed before starting clinical trials. This is the purpose of the present study.

Materials and methods

Materials

SPK-843 (batches P121, P122, P123, P124) was synthesized in the SPA Research Laboratories (SPA: Società Prodotti Antibiotici, Milan, Italy). It was used as such, with the addition of ascorbic acid and lactose (present as antioxidant and diluting agents in pharmaceutical formulation), or...
directly as lyophilized drug product having the following composition: SPK-843 25 mg, ascorbic acid 4.5 mg, lactose 250 mg. The Intralipid emulsions (10% and 20% commercial preparations) were supplied by Pharmacia & Upjohn (Nerviano, Italy).

L-(+)- ascorbic acid, lactose (monohydrate), dibasic sodium phosphate (dihydrate), citric acid (anhydrous) and glucose (anhydrous) met the EP and USP requirements.

The light source used in the stability under light tests was an ordinary neon lamp. Light intensity was measured with a Delta Ohm luxmeter HD 8366 type (Delta Ohm, Padova, Italy). The spectrophotometric analyses were performed with a Perkin Elmer Lambda 1 UV/VIS spectrophotometer (Perkin-Elmer, Milan, Italy), and the high-performance liquid chromatography (HPLC) analyses with a Waters apparatus (Waters, Vimodrone, Italy), made-up of a 2690 Separation unit, a 996 Diode Array Detector set at 378 nm and Millennium 2010 software. The apparatus was equipped with a Shiseido Capcell Pack C18 UG 120 column (5\(\mu\)m, 4.6 \(\times\) 150 mm) (Shisheido, Tokyo, Japan) with guard column, maintained at 40°C by an Alltech 530 column heater (ALLtech, Sedriano, Italy).

Animals

Male New Zealand albino rabbits (BMG, Civitate al Piano, BG, Italy and C. River of Calco, CO, Italy), aged 17–20 weeks and weighing 3.1–4.2 kg, were used in the venous toxicity tests. The randomization was performed using the random digits method.

The liquids to be tested were delivered through a B. Braun perfusing device (Braun, Milan, Italy).

Experiments were carried out following the Guiding Principles for the Care and Use of Laboratory Animals, the Recommendation from the Declaration of Helsinki and the European Community legislation n. 86/605.

Physical and chemical stability

The tests were performed with different SPK-843 concentrations in Intralipid emulsions. Ascorbic acid and lactose were also added at quantities of 0.18 and 10 mg, respectively, per 1 mg of SPK-843. To obtain the final alkaline pH value, the addition of small quantities (0.6–2 mg/mL) of dibasic sodium phosphate/citric acid buffer was often required.

The appearance of the emulsion was assessed with the naked eye and under the microscope immediately after its preparation and after 2 h at 20–25°C. The microscopic examination was performed at 2500×, the number and size of the larger oil droplets present in 40 microscopic fields (70 \(\times\) 55 \(\mu\)m) being recorded against Intralipid alone.

To assess SPK-843 dissolution in alkaline emulsions after 2 h at 20–25°C, half of the final emulsion was filtered through a Millipore HV membrane filter (0.45 \(\mu\)m) (Millipore, Milan, Italy), then the filter was visually controlled for undissolved drug substance (yellow in colour). In a few cases the SPK-843 dissolved in both filtered and unfiltered emulsions was also spectrophotometrically analysed by absorbance measurement at 378 nm; after the sample was diluted 1:50 with 95% ethanol, the results were expressed as the percentage of the value found before filtration.

Chemical stability tests were performed at natural and alkaline pH, both in the dark and in the light (1000 lux, measured close to the bottle containing the emulsions). Initially (0 h) and after 2 h at 25°C, the purity and quantity of SPK-843 were determined by HPLC. The mobile phase consisted of a mixture of (a) 100 mM diethylene/acetic
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acid solution at pH 10, and (b) acetonitrile (0–2 min 60% of A, 2–8 min linear gradient 60% to 50% of A, 8–10 min linear gradient 50% to 20% of A, 10–17 min 20% of A, 17–19 min linear gradient 20% to 60% of A, 19–22 min 60% of A) at a constant flow of 1 mL/min. Each time, a 2 mL sample was diluted to 10 mL with dimethylformamide (DMF) and 1 mL of internal standard solution (1 mg/mL of 4-nitroaniline in DMF). The mixture was shaken and centrifuged at 13 000 rpm for 5 min, then 10 μL were injected into the HPLC. The assay results at 2 h were expressed as the percentage of the value found at the initial time.

Venous toxicity

The tests were performed with different SPK-843 concentrations in Intralipid 10% emulsions or in 5% glucose solutions. To obtain the desired final pH values the addition of small quantities of 0.1 M HCl or dibasic sodium phosphate/citric acid buffer was required.

The resulting preparations were repeatedly perfused, at intervals of not less than 24 h, in the marginal ear vein of rabbits. The duration of each perfusion was always 30 min, whereas the volume administered, and consequently the dose/kg, varied depending on the test.

Administration was carried out for a total of 22 perfusions or up to vein occlusion due to phlebitis. The number of perfusions that it was possible to perform was taken as the venous tolerance index.

Statistical analysis

Analysis of variance was based on Student’s t-test.

Results and discussion

Physical and chemical stability

The physical stability was tested with Intralipid 10% and 20% emulsions. The 20% emulsion was discarded because with 0.5 mg/mL of SPK-843, clear indications of coalescence were seen upon microscopic examination.

The results of the physical stability tests with Intralipid 10% emulsions containing SPK-843 are summarized in Table 1. As can be seen, there were no problems of physical stability of the emulsion at mildly acid or basic pH and drug substance concentrations of 0.1–0.5 mg/mL, while 1 mg/mL concentrations produced oil flocculation.

Intralipid 10% emulsions containing 0.5 mg/mL were also prepared at the natural pH of 5.2–5.4 (no buffer added), using lyophilized drug product aged 24 months at 30°C and 60% relative humidity and 36 months at 25°C and 60% relative humidity and at 4°C. In these cases the active ingredient also dissolved completely and the emulsion was stable for not less than 2 h.

The chemical stability of SPK-843 solutions in Intralipid 10% emulsion, at natural and mildly basic pH, is reported in Table 2. Solutions of the drug substance (0.5 mg/mL) were perfectly stable for 2 h at 25°C even in well-lit rooms. Most likely, the opacity of the emulsion protects SPK-843 from light, as it is otherwise sensitive to violet and ultraviolet radiation.9

Venous toxicity

Interpretation of the venous toxicity data is complicated by the presence of several, partly interdependent, variables (concentration, volume administered, dose/kg of body weight, pH).

SPK-843, dissolved in Intralipid 10% emulsion, was definitely better tolerated (P < 0.05) than when dissolved in ordinary 5% glucose solution (Table 3, experiments 1–4). In the comparison the pH values differ; however, when using Intralipid 10% emulsion as the vehicle, a pH range of 5.3–7.6 proved to have no effect (Table 3, experiments 5–7). When the pH value increased to 8, venous toxicity increased as compared with pH 7.5 (P < 0.05) and pH 5.3 (P < 0.1).

The maximum number of infusions tolerated at pH 7.5

Table 1. Physical stability of Intralipid 10% emulsions containing different concentrations of SPK-843a

<table>
<thead>
<tr>
<th>SPK-843 conc. (mg/mL)</th>
<th>Buffer addition</th>
<th>pH</th>
<th>Naked eye exam.</th>
<th>Microscopic exam.</th>
<th>Undissolved drug substance (filtration)</th>
<th>% of dissolved drug substance (UV assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>no</td>
<td>7.3</td>
<td>satisfactory</td>
<td>satisfactory</td>
<td>not done</td>
<td>not done</td>
</tr>
<tr>
<td>0.25</td>
<td>no</td>
<td>6.2</td>
<td>satisfactory</td>
<td>satisfactory</td>
<td>not done</td>
<td>not done</td>
</tr>
<tr>
<td>0.5</td>
<td>no</td>
<td>5.4</td>
<td>satisfactory</td>
<td>satisfactory</td>
<td>not done</td>
<td>not done</td>
</tr>
<tr>
<td>0.5</td>
<td>yes</td>
<td>7.5</td>
<td>satisfactory</td>
<td>satisfactory</td>
<td>absent</td>
<td>99.9</td>
</tr>
<tr>
<td>0.5</td>
<td>yes</td>
<td>8</td>
<td>satisfactory</td>
<td>satisfactory</td>
<td>absent</td>
<td>100.2</td>
</tr>
<tr>
<td>1</td>
<td>no</td>
<td>4.7</td>
<td>satisfactory</td>
<td>flocculationb</td>
<td>not done</td>
<td>not done</td>
</tr>
</tbody>
</table>

aAscorbic acid and lactose (0.18 and 10 mg, respectively, per mg of SPK-843) were also present. Buffer was added to alkalize the pH of the more concentrated solutions.

bClusters of no measurable droplets; the solution became satisfactory after 1:1 dilution with water.
was closely related to the concentration and, above all, to the dose of SPK-843 dissolved in Intralipid 10% emulsion (Table 3, experiments 3, 6 and 8–12). This relationship with concentration (C) and dose (D) can be described approximately by the following equation, in which the coefficients of C and D reveal their different influence:

\[
I = \frac{21 - 2C - 6D}{210}
\]

where \( I \) = maximum number of infusions, \( C = \) SPK-843 concentration (mg/mL) and \( D = \) SPK-843 dose (mg/kg).

SPK-843 local tolerance in Intralipid 10% emulsion was also evaluated by histological examination (E. Ammannati & S. Peano, unpublished results). In the quoted work, the ear marginal veins at the site of the intravenous injection of three rabbits, treated with SPK-843 in 5% glucose solution (0.5 mg/mL, 1 mg/kg/day for 3–4 days) and in 10% Intralipid (0.5 mg/mL, 1 mg/kg/day for 8–14 days) were compared. Samples were embedded in paraffin blocks and c. 5 \( \mu \)m thick sections were obtained; slides were stained with haematoxylin and eosin, and examined under the light microscope. Increased frequency and/or degree of perivascular subacute inflammations and abscesses was seen in the glucose group compared with the Intralipid group. Epidermis ulcer, perivascular necrosis and histiocytosis were observed in the glucose group and not in the Intralipid group. This evaluation also confirmed the definite improvement in local tolerance obtained by using the lipid vehicle instead of 5% glucose.

Conclusions

The new polyene antibiotic SPK-843, when dissolved in Intralipid 10%, at pH 5.2–7.5 and at concentrations of 0.1–0.5 mg/mL, is physically and chemically stable for not less than 2 h, and induces no alterations of the emulsion. Under these conditions, the tolerance of venous infusion in rabbits is better than tolerance when dissolved in the more common 5% glucose solution. The results of tests in

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**Table 2.** Chemical stability of SPK-843 at different concentrations in Intralipid 10% emulsion

<table>
<thead>
<tr>
<th>SPK-843 conc. (mg/mL)</th>
<th>Buffer addition</th>
<th>pH</th>
<th>Light</th>
<th>0 h</th>
<th>2 h</th>
<th>HPLC assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>no</td>
<td>7.3</td>
<td>1000 lux</td>
<td>95.2</td>
<td>94.4</td>
<td>99.1</td>
</tr>
<tr>
<td>0.5</td>
<td>no</td>
<td>5.4</td>
<td>dark</td>
<td>95.7</td>
<td>95.9</td>
<td>99</td>
</tr>
<tr>
<td>0.5</td>
<td>no</td>
<td>5.4</td>
<td>1000 lux</td>
<td>95.4</td>
<td>95.5</td>
<td>99.9</td>
</tr>
<tr>
<td>0.5</td>
<td>yes</td>
<td>7.4</td>
<td>1000 lux</td>
<td>95.7</td>
<td>95.5</td>
<td>102.4</td>
</tr>
</tbody>
</table>

*Ascorbic acid and lactose were also present (0.18 and 10 mg, respectively, per mg of SPK-843). To alkalize the pH of the more concentrated solutions, 0.6 mg/mL of dibasic sodium phosphate/citric acid buffer was added.

**Table 3.** SPK-843 local tolerance after repeated 30 min infusions in rabbits

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Vehicle</th>
<th>Dose (mg/kg)</th>
<th>Conc. (mg/mL)</th>
<th>Volume (mL/30 min)</th>
<th>pH</th>
<th>Maximum no. of infusions</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5% glucose</td>
<td>1</td>
<td>0.5</td>
<td>6</td>
<td>5.3</td>
<td>3 ± 0.6</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>5% glucose</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>5.3</td>
<td>17 ± 0</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Intralipid</td>
<td>1</td>
<td>0.5</td>
<td>6</td>
<td>7.5</td>
<td>13 ± 6.7</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Intralipid</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>7.8</td>
<td>13 ± 2.1</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Intralipid</td>
<td>0.5</td>
<td>0.5</td>
<td>3</td>
<td>5.3</td>
<td>18 ± 6.9</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Intralipid</td>
<td>0.5</td>
<td>0.5</td>
<td>3</td>
<td>7.5</td>
<td>20 ± 3.5</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Intralipid</td>
<td>0.5</td>
<td>0.5</td>
<td>3</td>
<td>8.0</td>
<td>10 ± 2.6</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>Intralipid</td>
<td>0.2</td>
<td>0.1</td>
<td>6</td>
<td>7.5</td>
<td>22 ± 0</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>Intralipid</td>
<td>0.2</td>
<td>0.2</td>
<td>3</td>
<td>7.5</td>
<td>22 ± 0</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>Intralipid</td>
<td>1</td>
<td>0.1</td>
<td>30</td>
<td>7.5</td>
<td>16 ± 2.6</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>Intralipid</td>
<td>1</td>
<td>0.2</td>
<td>15</td>
<td>7.5</td>
<td>13 ± 1.5</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>Intralipid</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7.5</td>
<td>11 ± 2.6</td>
<td>3</td>
</tr>
</tbody>
</table>

*No addition of ascorbic acid and lactose. HCl (0.1 M) or buffer was added to obtain the desired final pH values of Intralipid emulsions containing SPK-843.

*Maximum number ± s.d.*
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the animal model, supported by histological data, indicate that doses up to 0.5 mg/kg can be administered for long periods of time. Even higher doses (1 mg/kg), probably excessive in clinical use, appear to be administrable.

References


Received 10 May 2001; returned 9 September 2001; revised 4 October 2001; accepted 5 November 2001