Factors Affecting the Release Rate of Terbutaline from Liposome Formulations After Intratracheal Instillation in the Guinea Pig

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Maximum duration of bronchodilator efficacy in inhaled liposome-based formulations depends on optimizing the in vivo release rate of the encapsulated bronchodilator. We investigated the effect of several formulation variables on the pulmonary residence time of \(^3\)H-terbutaline sulfate liposomes administered intratracheally in guinea pigs, using an improved method enabling the measurement of pulmonary drug absorption for extended periods of time in conscious animals. Half-lives of liposome-encapsulated \(^3\)H-terbutaline disappearance from the lungs and airways after instillation ranged from 1.4 to 18 hr and were markedly affected by liposome size, cholesterol content, and phospholipid composition. This study demonstrates that liposomes can significantly prolong the residence time of bronchodilators in the lungs and that precise control over the pulmonary residence time of encapsulated bronchodilators can be achieved by controlling formulation variables.

KEY WORDS: liposomes; bronchodilators; bioavailability; intratracheal instillation; pulmonary absorption; terbutaline.

INTRODUCTION

The development of liposomes as inhalable drug delivery systems has been reviewed by Mihalko et al. (1) and by Kellaway and Farr (2). The feasibility of using liposomes to achieve prolonged release of water soluble bronchodilators following administration to the lungs has recently been demonstrated (1). Measurements of pulmonary smooth muscle response to repeated histamine challenges in anesthetized guinea pigs indicated that liposome-encapsulated bronchodilators maintained bronchodilator activity longer than unencapsulated bronchodilators. These studies also demonstrated that cardiovascular side effects of these agonists were reduced by liposome encapsulation.

The rate at which drug becomes available from a prolonged-release formulation in the lungs may be critical in determining the duration of bronchodilator activity. It has been shown that for a given dose and minimum effective concentration, there is an optimum rate of drug release for a maximum duration of action (3). More rapid release results in a shorter duration of action, and if the drug becomes available too slowly, therapeutic levels are not reached at the effect site.

Formulation variables that influence the size, fluidity, and surface charge of liposomes are known to affect the release of liposome-encapsulated molecules (4,5). In addition, effects of drug-to-lipid ratio on the pharmacokinetics of liposome-encapsulated substances have been reported (6).

At present, in vitro test systems have not been developed which can accurately predict the rate of drug release from liposomes in vivo. For this reason, we studied the effects of phospholipid composition, cholesterol content, particle size, and drug-to-lipid ratio on the pulmonary kinetics of liposome-encapsulated drugs in the guinea pig, using an adaptation of the rat lung absorption model developed by Enna and Schanker (7).

MATERIALS AND METHODS

Formulations

Liposome-encapsulated \(^3\)H-terbutaline sulfate formulations in phosphate-buffered saline, pH 7.2, physiologic osmolality were made by thin-film hydration resulting in formation of multilamellar vesicles as previously described (8). \(^3\)H-Terbutaline sulfate (1.46 mCi/mg) was purchased from Amersham (Arlington Heights, IL). Dipalmitoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC), and terbutaline sulfate were supplied by Draco AB (Lund, Sweden). Dipalmitoyl phosphatidylglycerol (DPPG), distearoyl phosphatidylglycerol (DSPG), and egg phosphatidylglycerol (EPG) (all 99%) were obtained from Avanti Polar Lipids (Birmingham, AL), partially hydrogenated egg phosphatidylcholine (phEPC) was from Asahi Chemical Co. (Tokyo, Japan), and cholesterol, 99% (CH), was from Sigma Chemical Co. (St. Louis, MO).

After the initial formation of \(^3\)H-terbutaline liposomes, most formulations were extruded two to four times through 0.2-μm polycarbonate membranes to reduce the size of the liposomes and unencapsulated drug was removed by centrifugal pelleting followed by resuspension of the pellet in drug-free buffer. The mean particle size of extruded liposomes was determined by laser light scattering (Nicomf Laser Particle Sizer, Nicomp Instruments, Goleta, CA). The mean particle size of the unextruded liposome formulation was determined by Coulter Counter (Coulter Instruments, Hialeah, FL). The percentage encapsulation of \(^3\)H-terbutaline, determined by ultracentrifugation, was found to be between 94 and 100% for all formulations.

Intratracheal Instillation

The method of Enna and Schanker (7) for measurement of absorption rates of instilled compounds from the lungs of anesthetized rats was modified to allow measurements in conscious animals for periods of up to 48 hr after instillation.

Adult male Hartley guinea pigs (Harlan Sprague Dawley, Indianapolis, IN; weight range, 300–600 g) were anesthetized using a mixture of nitrous oxide (2 liters/min) and oxygen (0.9 liter/min) containing 5% isoflurane. Anesthetized animals were placed in a supine position on a 45° slanted support, and a small midline incision was made over the trachea. The trachea was exposed by blunt dissection of the sternohyoideus muscle. A small hole was made in the

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trachea between the fifth and the sixth tracheal rings using a 20-gauge needle. A short (10- to 15-cm) length of PE50 tubing was inserted into the hole and advanced to the bifurcation of the trachea. Solutions of ³H-terbutaline (300 µg/kg) or liposome-encapsulated ³H-terbutaline (300 µg terbutaline sulfate/kg, 1.5 µmol total lipid/kg) corresponding to approximately 1 ml/kg body weight were slowly instilled over a 1-min period using a 500-µl syringe attached to the PE50 tubing. Following instillation, the tubing was withdrawn and a small drop of cyanoacrylate adhesive was placed over the hole to seal the opening. The skin was closed with 3-0 Dexon sutures. The animal was removed from anesthesia and allowed to recover under a heating lamp. After recovery, animals were housed in individual plastic cages with access to food and water for the remainder of the study.

Tissue Samples

Groups of five guinea pigs were killed at various time points up to 48 hr after instillation for determination of the amount of ³H-terbutaline remaining in the lungs. The animals were anesthetized with gas anesthesia as described above and then killed with an intracardiac injection of 0.5 ml of sodium pentobarbital (65 mg/ml). The lungs and the portion of trachea below the instillation site were excised and homogenized in 10 ml of 75% acetonitrile for 45 sec (Brinkman Instruments Co., Westbury, NY). The homogenized lung sample was centrifuged for 10 min at 2500 rpm (Sorvall 6000, Du Pont Co., Wilmington, DE). A portion (1.0 ml) of the supernatant was transferred into a scintillation vial containing 10 ml of Ready Gel (Beckman Instruments, Fullerton, CA). A Packard 2000 beta counter (Packard Instrument Co., Downers Grove, IL) was used to determine tritium radioactivity (dpm). The lungs of an undosed animal were also excised and processed as above as an assay control blank.

As a recovery control, duplicate samples of each formulation were instilled into lungs removed from undosed animals, which were extracted with acetonitrile and counted as described above. The experimental samples were corrected for the observed recovery (80.4 ± 7.8%).

Calculations

The percentage of administered radioactivity remaining in the lungs was plotted versus time. Half-lives of ³H disappearance, with 95% confidence limits, were determined by monoexponential curve fits to the data using a nonlinear, weighted least-squares program (RSTRIP, MicroMath, Salt Lake City, UT).

RESULTS

Liposome Composition

Unencapsulated ³H-terbutaline left the lungs with a half-life of 1.3–1.4 hr, similar to the rate of terbutaline absorption reported after instillation of the drug to rats (9). The pulmonary kinetics of liposome-encapsulated ³H-terbutaline were formulation dependent (Table I, Fig. I). Disappearance of ³H-terbutaline encapsulated in DPPC/DPPG (95:5) liposomes was as rapid as unencapsulated ³H-terbutaline (half-life, 1.4 hr) but the addition of cholesterol (DPPC/DPPG/CH, 55:5:40) increased the half-life to 17.5 hr. A formulation containing egg phospholipids (EPC/EPC/CH, 55:5:40) had a half-life of 4.8 hr. Partial hydrogenation of the phospholipids in this formulation, from an iodine value of 60 to an iodine value of 40 (phEPC/EPC/CH, 55:5:40), increased the half-life slightly, to 5.4 hr. More complete hydrogenation of the egg phospholipids, to an iodine value of 1 (phEPC/EPC/CH), gave a half-life closer to that observed with fully saturated synthetic phospholipids (DSPC/DSPC/CH, 55:5:40, and DPPC/DPPG/CH, 55:5:40). Increasing the phospholipid acyl chain length from 16 carbons (DPPC) to 18 carbons (DSPC) did not appear to affect the observed kinetics.

Drug/Lipid Ratio

The half-life of liposome-encapsulated ³H-terbutaline was not affected by a fivefold change in drug/lipid ratio (0.04 to 0.2 mg drug/µmol lipid) for ³H-terbutaline in DPPC/DPPG (95:5) liposomes (Fig. 2).

Liposome Size

The residence time of ³H-terbutaline in unsized liposomes (mean particle size, 3900 nm) was longer than in smaller, extrusion-sized liposomes (mean particle size, 270 nm) of the same lipid composition (phEPC/EPC/CH, 55:5:40).

<table>
<thead>
<tr>
<th>No.</th>
<th>Lipid components*</th>
<th>Molar ratiob</th>
<th>Mean particle size (nm)</th>
<th>Half-life (hr)</th>
<th>95% confidence interval</th>
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<tr>
<td>1</td>
<td>Unencapsulated</td>
<td>—</td>
<td>—</td>
<td>1.4</td>
<td>0.8–2.5</td>
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<tr>
<td>2</td>
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<td>—</td>
<td>1.3</td>
<td>0.95–2.2</td>
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<tr>
<td>3</td>
<td>DPPC/DPPG</td>
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<td>1.4</td>
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<tr>
<td>4</td>
<td>EPC/EPC/CH</td>
<td>55:5:40</td>
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<td>4.8</td>
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</tr>
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<td>5</td>
<td>phEPC (IV40)/EPC/CH</td>
<td>55:5:40</td>
<td>270</td>
<td>5.4</td>
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<td>phEPC (IV40)/EPC/CH</td>
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<td>3900</td>
<td>14.5</td>
<td>13.3–16.0</td>
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<td>7</td>
<td>phEPC (IV1)/EPC/CH</td>
<td>55:5:40</td>
<td>270</td>
<td>16.3</td>
<td>15.2–17.5</td>
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<tr>
<td>8</td>
<td>DPPC/DPPG/CH</td>
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<td>55:5:40</td>
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<td>17.9</td>
<td>14.4–23.6</td>
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</table>

* IV, iodine value. See text for other abbreviations.

b Approximately 0.2 mg terbutaline/µmol lipid in all liposome formulations.