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

Robert M. Fielding[1] and Robert M. Abra[1,2]

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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, physiological 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 pelletting followed by resuspension of the pellet in drug-free buffer. The mean particle size of extruded liposomes was determined by laser light scattering (Nicomp 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

[1] Liposome Technology, Inc., 1050 Hamilton Court, Menlo Park, California 94025.

[2] To whom correspondence should be addressed.

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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 ^{3}H-terbutaline (300 μg/kg) or liposome-encapsulated ^{3}H-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 ^{3}H-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, Dupont 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 ^{3}H 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 ^{3}H-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 ^{3}H-terbutaline were formulation dependent (Table I, Fig. 1). Disappearance of ^{3}H-terbutaline encapsulated in DPPC/DPPG (95:5) liposomes was as rapid as unencapsulated ^{3}H-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/EPG/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/EPG/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/EPG/CH), gave a half-life closer to that observed with fully saturated synthetic phospholipids (DSPC/DSPG/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 ^{3}H-terbutaline was not affected by a fivefold change in drug/lipid ratio (0.04 to 0.2 mg drug/μmol lipid) for ^{3}H-terbutaline in DPPC/DPPG (95:5) liposomes (Fig. 2).

Liposome Size

The residence time of ^{3}H-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/EPG/CH,

Table I. Composition, Size, and Pulmonary Disappearance Half-Lives of Liposome-Encapsulated ^{3}H-Terbutaline Formulations Administered by Intratracheal Instillation (300 μg/kg) to Guinea Pigs

No.	Lipid components[a]	Molar ratio[b]	Mean particle size (nm)	Half-life (hr)	95% confidence interval
1	Unencapsulated	—	—	1.4	0.8–2.5
2	Unencapsulated	—	—	1.3	0.95–2.2
3	DPPC/DPPG	95:5	207	1.4	0.95–2.9
4	EPC/EPG/CH	55:5:40	238	4.8	4.4–5.3
5	phEPC (IV40)/EPG/CH	55:5:40	270	5.4	4.8–6.1
6	phEPC (IV40)/EPG/CH	55:5:40	3900	14.5	13.3–16.0
7	phEPC (IV1)/EPG/CH	55:5:40	270	16.3	15.2–17.5
8	DPPC/DPPG/CH	55:5:40	243	17.5	11.7–34.5
9	DSPC/DSPG/CH	55:5:40	231	17.9	14.4–23.6

[a] IV, iodine value. See text for other abbreviations.
[b] Approximately 0.2 mg terbutaline/μmol lipid in all liposome formulations.

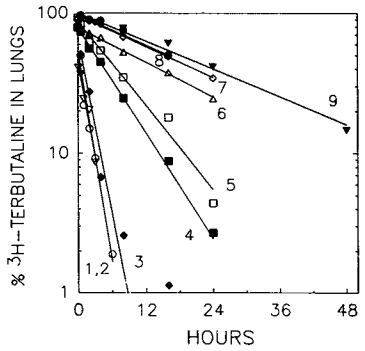

Fig. 1. Disappearance of radioactivity from the lungs after intratracheal instillation of liposome-encapsulated (3–9) and unencapsulated (1–2) ^{3}H-terbutaline. Formulations are numbered as in Table I.

55:5:40). The unextruded formulation had a half-life of 14.5 hr, compared to 5.4 hr for the extruded formulation (Table I).

DISCUSSION

These studies demonstrate that the kinetics of liposome-encapsulated ^{3}H-terbutaline in the lungs are sensitive to both the composition and the size of the liposomes used. The presence of phospholipids with saturated hydrocarbon chains increased the drug residence time in the lungs, as did cholesterol. These components are known to decrease liposome membrane permeability to solutes and to protect liposomes from *in vivo* destabilization (8). Manipulation of the cholesterol content alone allowed the measured pulmonary half-life of ^{3}H-terbutaline to be increased nearly 10-fold. In addition, it was shown that large, unsized liposomes were more effective at prolonging the residence time of ^{3}H-terbutaline in the lungs than small, extruded liposomes of the same lipid composition. This effect may be attributed to a reduced number of lamellae in the membranes of extruded liposomes, increasing solute permeability, or size effects on the distribution and disposition of liposomes in the lungs and airways.

A fivefold change in the drug/lipid ratio had no effect on the clearance of encapsulated ^{3}H-terbutaline in DPPC/DPPG liposomes in this study, indicating that variations in drug/lipid ratio may have little effect on the performance of liposome-encapsulated bronchodilators. The lower drug/lipid ratio formulation simply encapsulated a drug solution at a lower concentration than the standard concentration utilized throughout these experiments. Thus, diffusion across the liposomal membrane might reasonably be expected to remain the same, the major change being an increased dose of lipid to the lung in order to carry the same quantity of drug. This apparently had no effect upon overall rate of drug loss from the lung.

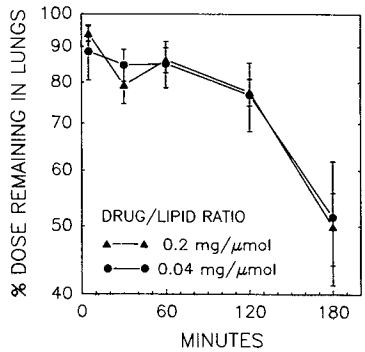

Fig. 2. Effect of drug/lipid ratio on the pulmonary kinetics of liposome-encapsulated ^{3}H-terbutaline. Lipid composition, DPPC/DPPG (95:5); terbutaline dose, 300 μg/kg. Mean values ± SD (n = 5).

In this study, extractable ^{3}H-radioactivity was measured only in the lungs. However, since it has been shown that terbutaline is not metabolized in the guinea pig lung (10) or degraded by liposomes (data not shown), it is likely that the losses of radioactivity measured in this study represent systemic absorption of ^{3}H-terbutaline from the lungs, possibly modified by some degree of mucociliary transport. The fact that radiolabel remained in the lungs for as long as 48 hr after instillation and was strongly affected by liposome encapsulation and liposomal lipid composition suggests that at least some of the intratracheally instilled liposomes were not subject to rapid mucociliary clearance and could be used as a long-term sustained-release depot for local or systemic delivery of therapeutic agents. Potent drugs with short elimination half-lives and poor oral availability such as polypeptides and nucleotides would be among potential candidates for such a delivery system.

Different portions of the bronchopulmonary tree possess different characteristics; it is possible that drug release from liposomes is affected by the distribution of formulation achieved during administration and later altered by mucociliary transport and other mechanisms. Animal studies to date have utilized instillation of liquid formulations in order to obtain accurate dosimetry. Such results are dependent upon the spreading of the instilled dose within the lung and their interpretation may be complicated by the presence of components capable of affecting the spreading process.

The distribution and absorption of inhaled aerosols in the lungs and airways are different from those of instilled liquids (11–13), and it is possible that release kinetics of aerosol formulations in humans will differ considerably from release kinetics of instilled formulations in animals. In addition, the size and aerodynamic properties of human airways may result in a significantly different distribution and rehydration of aerosolized liposomes compared to rodent test systems, which may affect observed release kinetics, duration, onset, and intensity of effect. Severe bronchoconstriction in asthmatics may also lead to altered distribution and product performance compared to normals.

While more work is needed to extrapolate these findings to inhaled liposome formulations in humans, the present study clearly indicates the important role of certain formulation variables in obtaining liposome-encapsulated formulations with optimal prolonged-release performance characteristics. The sensitivity of performance to liposome composition also implies that relatively small formulation changes could significantly alter the characteristics of liposome-encapsulated bronchodilators.

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