

Effect of Dose and Release Rate on Pulmonary Targeting of Liposomal Triamcinolone Acetonide Phosphate

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Purpose. To demonstrate the importance of dose and drug release rate for pulmonary targeting of inhaled glucocorticoids using an animal model of intrapulmonary drug deposition.

Methods. Liposomes composed of 1,2-distearoyl phosphatidylcholine (DSPC), 1,2-distearoyl phosphatidylglycerol (DSPG) and triamcinolone acetonide phosphate (TAP) or liposomes containing triamcinolone acetonide (TA) were prepared by a mechanical dispersion method followed by extrusion through polycarbonate membranes. Encapsulation efficiency was assessed after size exclusion gel chromatography by reverse phase HPLC. The effect of liposome size (200 nm and 800 nm) on the release kinetics of water-soluble encapsulated material was determined *in vitro* at 37°C using 6-carboxyfluorescein as a marker and Triton X-100 (0.03%) as a leakage inducer. To investigate the relationship between drug release and pulmonary targeting, 100 µg/kg of TAP in 800 nm liposomes was delivered to male rats by intratracheal instillation (IT) and the results compared to data for 100 µg/kg TA liposomes (recently shown to exhibit a rapid drug release under sink conditions) and to previous studies reported for an equal dose of TAP in solution and TAP in 200 nm (1). Pulmonary targeting was assessed by simultaneously monitoring glucocorticoid receptor occupancy over time in lung and liver using an *ex vivo* receptor binding assay as a pharmacodynamic measure of glucocorticoid action. To assess the effect of dose on pulmonary targeting experiments were performed using 2.5, 7.5, 25, 100, and 450 µg/kg of TAP in 800 nm liposomes.

Results. The *in vitro* efflux of 6-carboxyfluorescein from (DSPC: DSPG) liposomes after exposure to Triton-X was biexponential. The terminal half-lives of 3.7 h and 9.0 h for the 200 nm and 800 nm liposomes, respectively, demonstrated that larger liposomes promote slower release of encapsulated water-soluble solute while previous results already indicated that encapsulation of lipophilic TA does not result in sustained release. Pulmonary targeting, defined as the difference between cumulative lung and liver receptor occupancies was most pronounced for the 800 nm liposomes (370%*h), followed by the 200 nm preparation (150%*h). No targeting was observed for TAP in solution (30%*h) or the rapid releasing TA liposome preparation. Correspondingly, the mean pulmonary effect time (MET) increased from 2.4–3.0 hr for TA liposomes or TAP in solution to 5.7 h and >6.2 h for TAP in 200 nm and in 800 nm liposomes, respectively. Escalating doses of TAP encapsulated in 800 nm liposomes revealed a distinct bell shaped relationship between the TAP dose and pulmonary targeting with a maximum occurring at 100 µg/kg (370%*h).

Conclusions. The *in vivo* data presented here confirm that pulmonary residence time and dose affect the extent of lung targeting of glucocorticoids delivered via the lung.

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INTRODUCTION

Inhaled glucocorticoids are the drugs of choice for the treatment of chronic asthma as aerosols target the lung at the site of action, and, in addition, because local delivery may reduce systemic side effects. However, inhaled glucocorticoids exhibit systemic side effects (2) and a further improvement of pulmonary targeting of inhaled glucocorticoids is desirable.

Different approaches have been taken to optimize pulmonary targeting which include the development of drugs with high clearance and low oral bioavailability (3). Alternatively, sustained release formulations, such as liposomal drug carrier systems, have been suggested to prolong pulmonary drug action (1,4,5). However, detailed information on the effect of release rate on pulmonary targeting is not available. Recently, we have developed a mathematical model which predicts pulmonary selectivity of inhaled glucocorticoids by taking into account the pharmacokinetic/pharmacodynamic properties of these drugs (6). Extrapolations from this theoretical model suggest that drug release rate and dose are factors which can modulate the extent of pulmonary targeting.

In the present study, an animal model (1,7) was used to assess the effect of release rate and dose on pulmonary targeting of glucocorticoids upon intratracheal instillation of liposome preparations with different release rate characteristics and different doses of encapsulated material.

MATERIALS AND METHODS

Chemicals

Triamcinolone acetonide 21-phosphate dipotassium salt was donated by Bristol Myers Squibb (Neu-Isenburg, Germany). [6,7-³H(N)]-triamcinolone acetonide (35.4 Ci/mmol) was purchased from DuPont NEN® Research Products (Boston, MA). Phospholipids (purity >99%), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-distearoyl-sn-glycero-3-{phospho-rac-(1-glycerol)} (DSPG) were purchased from Avanti Polar Lipids (Alabaster, AL). 6-carboxyfluorescein was purchased from Sigma (St. Louis, MO). All other reagents were of analytical grade.

Animals

Male F-344, non-adrenalectomized rats weighing approximately 200–250 g were obtained and maintained as described in (1).

Preparation of TAP-liposomes

A modification of a previously employed method (1) was used to prepare multilamellar vesicles containing triamcinolone acetonide phosphate (TAP). A chloroform solution of DSPC

(89.4 mg) and DSPG (10.1 mg) was placed in a round bottom flask. The organic solvent was removed under vacuum at 63°C until a dry film was obtained and 1 ml of 60 mg/ml of TAP in isotonic phosphate buffer saline (PBS) was added. The drug and lipids were dispersed mechanically for 1 h at 63°C. After 5 freeze/thaw cycles, the vesicles were extruded through 800 nm polycarbonate membrane filters (Poretics, Co., Livermore, CA) using a stainless-steel metallic extruder (Lipex Biomembranes Inc., Vancouver, B.C.). The resulting vesicles were separated from non-encapsulated drug by size exclusion chromatography (Sephadex G-50) and characterized for size (volume weighted mean diameter), encapsulation efficiency and osmolarity as described in (1). The lipid content of the liposomal preparations was determined by a colorimetric method (10).

Preparation of 6-carboxyfluorescein (CF) Liposomes

CF was selected as a surrogate solute marker to be encapsulated in multilamellar vesicles because like TAP, it is a water soluble substance and would be expected to partition similarly within the liposomal matrix. One ml of 200 mM 6-carboxyfluorescein (purified according to (8)), was added to the dried film of phospholipids. The mixture was dispersed, extruded through 200 nm or 800 nm polycarbonate filters and processed as described for TAP-liposomes.

In Vitro Release Rate Studies

The effect of liposome size on the release rate of entrapped material was tested under sink conditions using 6-carboxyfluorescein as a marker and Triton X-100 (0.03%) as a leakage inducer.

Purified CF-liposomes (equivalent to 6 μ M total phospholipid) were diluted 3-fold with PBS, followed by addition of Triton X-100 (0.03% final concentration). After vortexing, 200 μ l aliquots of each sample were transferred into 96-well plates and incubated at 37°C. Carboxyfluorescein efflux was monitored close to wavelengths of maximum excitation and emission over 5 h on a luminescence Spectrophotometer Perkin Elmer model LS50B with an excitation wavelength of 470 nm and emission wavelength of 520 nm. A blank value was obtained by carrying out the experiment in the absence of Triton X-100. The 100% fluorescence was determined after addition of 50 μ l of Triton X-100 to each sample. The percentage of carboxyfluorescein released was calculated as previously described (11).

The percentage of carboxyfluorescein encapsulated versus time was fitted to a biexponential equation (Scientist, Micro-math, Salt Lake City, UT) and the half-life of release was calculated from the initial and terminal phases.

Animal Experiments

Animals were anesthetized via intraperitoneal injection with a 0.2–0.5 ml of a mixture of xylezine (20 mg/ml), ketamine (100 mg/ml) and acepromazine (10 mg/ml) and TAP in 800 nm liposomes (TAP-lip 800 nm; equivalent to 100 μ g/kg of TAP) were administered by intratracheal instillation as previously described (1). Animals (one animal per time point) were decapitated at 1, 2.5, 6, 12 or 18 hours after intratracheal (IT) administration of the preparation, and the lungs (exclusive of trachea and mainstem bronchi) and livers were immediately processed for receptor binding studies (1). Trachea and main-

stream bronchi were not used as it is difficult to homogenize and the receptor density in preliminary experiments was very low.

A total of 3 independent experiments were performed on different days. TA in 200 nm liposomes (TA-lip) was administered in the same fashion and animals were decapitated at 0.25, 1, 3 and 6 h.

To test for the effect of dose on pulmonary targeting, experiments were performed using doses of 2.5, 7.5, 25, 100 or 450 μ g/kg of TAP-lip 800 nm, respectively.

Receptor Binding Studies

A previously described receptor-binding assay was employed (1) to monitor free cytosolic receptors. In the current study, one high concentration of 3 H-triamcinolone acetonide (30 nM 3 H-TA in the presence and absence of high concentrations of unlabeled TA, 3 μ M) was used to determine the maximum specific binding (B_{max}). This was justified by the comparison of calculated B_{max} values using this method and the previously employed Scatchard method ($R^2 = 0.985$, $n = 45$) (1).

Data Analysis

To test for differences in receptor occupancy between lung and liver the pool of paired hepatic and pulmonary receptor occupancies of all individual time points were compared by paired t-test as described previously (1) assuming $p < 0.05$.

To quantify the degree of pulmonary targeting, the cumulative change from baseline (AUC) was calculated for the investigation period by the trapezoidal rule from percent occupied receptor time profiles for both the local and systemic organs. Pulmonary targeting (PT) was defined as

$$PT = AUC_{Lung} - AUC_{Liver} \quad (1)$$

For comparison, the previously employed (1) targeting index ($PT = AUC_{Lung}/AUC_{Liver}$) was also derived. Differences in targeting among TAP-lip 800 nm, TAP-lip 200 nm and TAP-sol and among doses, were tested for significance using Tukey's multiple comparison test assuming $\alpha < 0.05$. The mean effect time was calculated as previously described (1). In instances where the effect (receptor levels) did not return to baseline, MET was calculated from the data without extrapolation.

RESULTS

Characterization of Liposomes

The volume weighted mean particle diameter (mean diameter \pm S.D.) of the liposomal TAP preparations resulting from the Gaussian distribution analysis was 220 ± 18 nm ($n = 10$) for the 200 nm CF-liposomes and 760 ± 90 nm ($n = 21$) for the 800 nm TAP-lip and CF-liposomes with 70% of the initial amount of lipid incorporated into the vesicles. The encapsulation efficiency for TAP-lip 800 nm was $16.5 \pm 2.1\%$ ($n = 18$). Characterization of TA liposomes (200 nm particle diameter) has been described elsewhere (9).

In Vitro Release Studies

A semilogarithmic plot of % carboxyfluorescein encapsulated versus time showed a biexponential decay for both lipo-

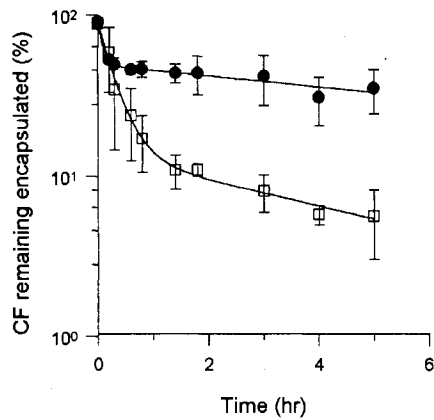


Fig. 1. *In vitro* release studies of DSPC:DSPG CF-liposomes at 37°C under sink conditions as a function of liposome size: 200 nm diameter (\square), 800 nm diameter (\bullet). The percentage of 6-carboxyfluorescein remaining encapsulated over time of incubation is shown. Error bars represent mean \pm S.D. for $n = 3$.

some sizes (Figure 1). The CF-lip 200 nm released about 80% of CF with a half-life of 23 min while the remaining 20% was released with a half-life of 3.7 h. CF-lip 800 nm showed a rapid initial phase for approximately 10% of the CF ($t_{1/2}$ 4 min) with a more prolonged terminal phase ($t_{1/2}$ 9.0 h) for the remaining 90% of CF released. TA liposomes released 90% of drug load within 2-3 minutes (9).

Receptor Binding

Time courses of free glucocorticoid receptors in lung and liver after intratracheal administration of 100 $\mu\text{g}/\text{kg}$ TAP-lip 800 nm are compared in Figure 2 to TA-lip and to previous data obtained for equivalent doses of TAP in solution (TAP-sol) and TAP in 200 nm liposomes (TAP-lip 200 nm) (1).

Pulmonary targeting (PT), defined as the difference between cumulative lung and liver receptor occupancies was

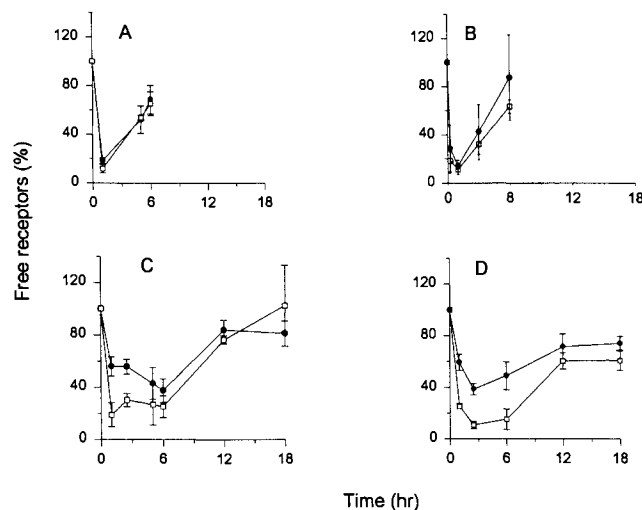


Fig. 2. Lung (\square) and liver (\bullet) glucocorticoid receptor occupancy profiles after intratracheal instillation of 100 $\mu\text{g}/\text{kg}$ of: A) TAP-sol from (1); B) TA-lip, C) TAP-lip 200 nm from (1); and D) TAP-lip 800 nm. Error bars represent mean \pm S.D. for $n = 3$ to 6.

most pronounced for the slowest release preparation (PT_{TAP-lip 800 nm} = 370 %*h, $p < 0.02$), followed by the 200 nm preparation (PT_{TAP-lip 200 nm} = 150 %*h, $p < 0.02$). No pulmonary targeting was observed for TAP in solution (PT_{TAP-sol} = 30 %*h, $p > 0.2$; Figure 2A) or TA-lip (PT_{TA-lip} = -60 %*hr, $p > 0.07$; Figure 2B). Multiple comparison Tukey's tests showed significant differences among the tested preparations. The mean effect times (MET; refer to Table 1) correlated well with the *in vitro* release studies; these studies demonstrated that by increasing liposome size, both *in vitro* stability (Figure 1) and mean effect time were increased.

The administration of escalating doses of TAP-lip 800 nm revealed a distinct bell shaped relationship between the TAP dose and pulmonary targeting with a maximum pulmonary targeting occurring at 100 $\mu\text{g}/\text{kg}$ (PT_{TAP-lip 800 nm} = 370%*h) (Figure 3, Table 2). Significant differences in receptor occupancy between organs were found for all doses except for 2.5 $\mu\text{g}/\text{kg}$ and 450 $\mu\text{g}/\text{kg}$. Tukey's test multiple comparison applied to means of pulmonary targeting as a function of dose revealed significant difference between 100 $\mu\text{g}/\text{kg}$ and the rest of the doses, while no significant difference was found among 7.5, 25 and 450 $\mu\text{g}/\text{kg}$, but between these three doses and 2.5 $\mu\text{g}/\text{kg}$.

DISCUSSION

We have recently expressed pulmonary targeting as the ratio of cumulative receptor occupancy in lung and liver (AUC_{Lung}/AUC_{Liver}; (1)). However, computer simulations (6) have shown that pulmonary targeting defined in these terms reaches its maximum as the dose approaches zero (see also Table 2) and we believe now that pulmonary targeting is more accurately defined as the difference between lung and liver receptor occupancies (AUC_{Lung}-AUC_{Liver}), especially when the pulmonary selectivity is evaluated as a function of dose (see Table 2 for comparison of both parameters).

Size-dependent leakage from liposomes has been previously reported after pulmonary instillation (12). In agreement with this, the larger multilamellar liposomes (800 nm) released the encapsulated material with longer terminal half-life than smaller vesicles (200 nm) (Figure 1). In addition, 200 and 800 nm liposomes showed a biexponential release characteristic. Biexponential release characteristics are well described for the interaction of liposomes with plasma (13) and have been linked to a rapid rise in liposome membrane permeability because of a localized change of the liposome surface tension at the surface of the liposome. It is very likely that the Triton X100 is inducing a similar change in permeability. Clearly, this would explain the more pronounced release from the 200 nm liposomes as the liposomal surface area is larger than that of the 800 nm preparation. A resulting rapid initial drug release may be therapeutically advantageous since under these conditions a more rapid onset of drug action in the lung could be attained, followed by a prolonged release for the remainder of the dosage form.

Computer simulations and animal experiments have shown the advantage of controlling the drug release rate as a mean of increasing lung residence time upon intratracheal instillation (12,14). In addition, it has been suggested, but not clearly demonstrated, that pulmonary selectivity depends on a slow release of the drug from the delivery form (1,4,7). We have shown that the IT administration of slow release triamcinolone acetate phosphate liposomes (TAP-lip 800 nm) resulted in

Table 1. Cumulative Receptor Occupancy (AUC), Pulmonary Targeting and Mean Pulmonary Effect Times (MET) after Intratracheal Administration of TAP in Solution

	TAP-sol ^a	TA-lip 200 nm ^b	TAP-lip 200 nm	TAP-lip 800 nm
	Cumulative change from baseline (AUC %*hr)			
Lung	370 ± 50	320 ± 85	770 ± 120	1070 ± 70
Liver	340 ± 40	380 ± 10	620 ± 150	700 ± 140
Pulmonary Targeting (%*h) (AUC _{lung} - AUC _{liver})	30 ± 10	-60 ± 80	150 ± 60	370 ± 70
Pulmonary Targeting (%*h) (AUC _{lung} /AUC _{liver})	1.0	0.85	1.2	1.5
Mean Pulmonary Effect Time (h)	3	2.4	5.7	>6.2

^a TAP-sol (immediate release; 100 µg/kg), TA in 200 nm liposomes (100 µg/kg).

^b TAP in 200 nm liposomes (TAP-lip 200 nm, intermediate release; 100 µg/kg) and TAP in 800 nm liposomes (TAP-lip 800 nm, slow release; 100 µg/kg).

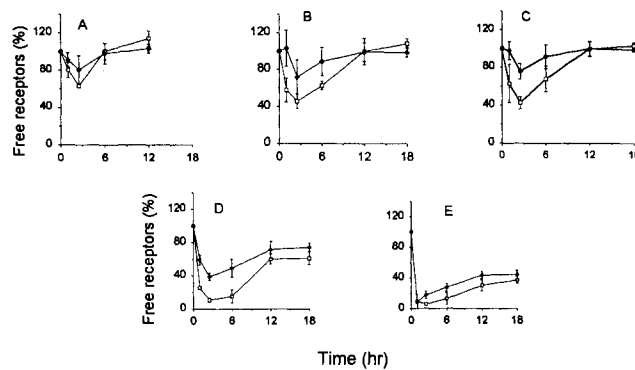


Fig. 3. Lung (□) and liver (◆) glucocorticoid receptor occupancy profiles after intratracheal instillation of escalating doses of TAP in 800 nm liposomes: A) 2.5 µg/kg, n = 4; B) 7.5 µg/kg, n = 3; C) 25 µg/kg, n = 3; D) 100 µg/kg, n = 3; and E) 450 µg/kg, n = 3. Error bars represent mean ± S.D.

higher pulmonary selectivity (Figure 2, Table 1) as well as in a more prolonged pulmonary effects (Figure 2D; see MET Table 1) than the IT administration of intermediate release rate liposomes (TAP-lip 200 nm; Figure 2C) or the immediate release TAP solution (TAP-sol; Figure 2A), indicating the importance of the release rate for pulmonary targeting. Intratracheal (IT) administration of TA-lip (100 µg/kg of TA) resulted

in a lack of targeting (Figure 2B) with non-significant differences between pulmonary and hepatic receptor occupancy. This finding is in agreement with the unhindered release of the lipophilic TA from the liposome matrix upon dilution *in vitro* (9), suggesting that the sole administration of liposomal encapsulated drug is not sufficient for attaining pulmonary targeting. These experiments consequently illustrate a potential pitfall which is likely to occur whenever lipophilic, membrane-penetrable glucocorticoids are selected for liposomal-based tissue targeting.

The relationship between pulmonary targeting and release rate does not imply that the increase in pulmonary targeting is because of decreased systemic side effects. Although pulmonary receptor occupancies relatively increased as the release rate decreased (compare TAP solution, TAP 200 nm and TAP 800 nm liposomes in Table 1), liver receptor occupancy also increased (Table 1). This is not surprising since a slower absorption has been shown to increase the cumulative systemic effect for a given dose (15). Our experiments, however, indicate that the increase in pulmonary effects was more pronounced and, consequently pulmonary selectivity was enhanced. This can be rationalized as the free (pharmacodynamically active) drug concentration in the lung exceeded that in the systemic circulation (liver) over a prolonged period of time when the drug release rate was slowed down. The clinical relevance of these findings is that by administering glucocorticoids in slow release

Table 2. Cumulative Receptor Occupancy (AUC), Pulmonary Targeting and Mean Pulmonary Effect Times (MET) After Intratracheal Administration of Escalating Doses of TAP in 800 nm Liposomes

	Dose (µg/kg)				
	2.5	7.5	25	100	450
	Cumulative change from baseline (AUC %*hr)				
Lung	90 ± 30	340 ± 130	345 ± 110	1070 ± 70	1260 ± 160
Liver	78 ± 30	110 ± 85	130 ± 50	700 ± 140	1080 ± 90
Pulmonary Targeting (%*hr) AUC _{lung} - AUC _{liver}	14 ± 40	230 ± 70	215 ± 50	370 ± 70	140 ± 100
Pulmonary Targeting (%*hr) AUC _{lung} /AUC _{liver}	1.2	3.0	2.7	1.5	1.2
Mean Pulmonary Effect Time (hr)	2.5	3.9	4.0	>6.2	>8.1

preparations, the frequency of administration as well as the dose can be decreased possibly leading to a reduction of systemic side effects while therapeutically desirable pulmonary effects are sustained.

Mucociliary clearance and macrophage phagocytic uptake of particles are normally operant and represent independent clearance mechanism for particles deposited in the lung. It could be argued that differences in mucociliary clearance and/or phagocytic uptake rather than differential release characteristics might account for differences observed in pulmonary targeting. Because of the small size of our liposomes (<1 μm), it is unlikely that that size-dependent mucociliary clearance or deposition after inhalation or instillation could have accounted for differences in pulmonary targeting, as both factors have been found to be independent of liposome size, and in the case of inhalation, depend on the droplet (not liposome size) of the aerosolized substance (16). It is also unlikely that the magnitude of differences we observed in pulmonary targeting could be accounted for by differential rates of phagocytic uptake of liposomes by lung macrophages. We base this assumption on uptake studies using CF-loaded liposomes and THP-1 macrophage monolayers (a human derived macrophage cell line). These experiments revealed that 99.8 and 99.5% of liposomal CF was not taken up by macrophages after a 2 hour incubation at 37°C with 200 and 800 nm CF-loaded liposomes, respectively (RGR unpublished observations). These results were obtained even in the absence of glucocorticoids which slow down the phagocytic activity of macrophages (17).

The administration of escalating doses of TAP-lip 800 nm showed a typical bell shaped curve with a maximum at 100 $\mu\text{g}/\text{kg}$ (Table 2), and a significant difference between pulmonary and hepatic receptor occupancy increased until a maximum was reached (Figure 3 panels B, C, D). At low doses, pulmonary and systemic receptors are hardly occupied with small differences between pulmonary and systemic receptors. Pulmonary receptors are getting saturated at high doses and at a certain point, a further increase in dose will not result in a further increase in receptor occupancy. However, more drug will enter the systemic circulation, resulting in an increase in the systemic receptor occupancy and a loss of pulmonary targeting. Thus, both low (2.5 $\mu\text{g}/\text{kg}$) and high (450 $\mu\text{g}/\text{kg}$) doses of TAP resulted in close superimposition of lung and liver receptor occupancy and, consequently, in low pulmonary targeting (Figure 3A, 3E). These experiments suggest there is a dose "optimum" for which maximum pulmonary selectivity is observed. Although it is recognized that a dose optimum might not necessarily be directly indicative of clinical response in asthma of varying severity, our findings clearly show that overdosing and "under"-dosing will always go parallel with a decreased pulmonary targeting.

Recent approaches to increase pulmonary selectivity of glucocorticoids have included the development of drugs with higher systemic clearance and lower oral bioavailability. This study together with previously derived computer simulations (6) suggest that drug release rate and dose should also be

considered during drug development and for evaluating dosage regimens with minimized systemic side effects.

REFERENCES

1. R. Gonzalez-Rothi, S. Suarez, G. Hochhaus, H. Schreier, A. Lukyanov, H. Derendorf, and T. Dalla-Costa. Pulmonary targeting of liposomal triamcinolone acetonide phosphate. *Pharm. Res.* **13**:1699-1703 (1996).
2. W. H. Nicolaizik, J. L. Marchant, M. A. Preece, and J. O. Warner. Endocrine and lung function in asthmatic children on inhaled corticosteroids. *Am. J. Respir. Crit. Care Med.* **150**:624-628 (1994).
3. M. Johnson. Pharmacodynamics and pharmacokinetics of inhaled glucocorticoids. *J. Allergy Clin. Immunol.* **97**:169-176 (1996).
4. R. Brattsand and B. I. Axelsson. Basis of airway selectivity of inhaled glucocorticoids. In R. P. Schleimer, W. W. Busse, and P. M. O'Byrne (eds.), *Inhaled glucocorticoids in asthma*, Marcel Dekker, New York, US, 1997, pp. 351-379.
5. V. Vidgren, J. C. Waldrep, J. Arppe, M. Black, J. A. Rodarte, W. Cole, and V. Knight. A study of $^{99\text{m}}$ technetium-labelled beclomethasone dipropionate dilaurylphosphatidylcholine liposome aerosol in normal volunteers. *Int. J. Pharm.* **115**:209-16 (1995).
6. G. Hochhaus, H. Möllmann, H. Derendorf, and R. Gonzalez-Rothi. Pharmacokinetic/pharmacodynamic aspects of aerosol therapy using glucocorticoids as a model. *J. Clin. Pharmacol.* **37**:881-892 (1997).
7. G. Hochhaus, R. Gonzalez-Rothi, A. Lukyanov, H. Schreier, H. Derendorf, and T. Dalla-Costa. Assessment of glucocorticoid lung targeting by ex-vivo receptor binding studies. *Pharm. Res.* **12**:134-137 (1995).
8. E. Ralston, L. M. Hjelmeland, R. D. Klausner, J. N. Weinstein, and R. Blumenthal. Carboxyfluorescein as a probe for liposome-cell interactions effect of impurities, and purification of the dye. *Biochim. Biophys. Acta* **649**:133-137 (1981).
9. H. Schreier, A. N. Lukyanov, G. Hochhaus, and R. J. Gonzalez-Rothi. Thermodynamic and kinetic aspects of the interaction of triamcinolone acetonide with liposomes. *Proceed. Inter. Symp. Control. Rel. Bioact. Mater.* **21**:228-229 (1994).
10. J. C. M. Stewart. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal. Chem.* **104**:10-14 (1980).
11. Y. Liu and L. Regen. Control over vesicle rupture and leakage by membrane packing and by the aggregation state of an attacking surfactant. *J. Am. Chem. Soc.* **115**:708-713 (1992).
12. R. M. Fielding and R. M. Abra. Factors affecting the release rate of terbutaline from liposome formulations after intratracheal instillation in the guinea pig. *Pharm. Res.* **9**:220-223 (1992).
13. C. A. Hunt. Liposome Disposition in vivo: V. Liposome stability in plasma and implications for drug carrier function. *Biochim. Biophys. Acta*, **719**, 450-463 (1982).
14. I. Gonda. Drugs administered directly into the respiratory tract: modeling of the duration of effective drug levels. *J. Pharm. Sci.* **77**:340-346 (1988).
15. G. Hochhaus and H. Derendorf. Dose optimization based on pharmacokinetic/pharmacodynamic modeling. In H. Derendorf and G. Hochhaus (eds.), *Handbook of pharmacokinetic/pharmacodynamic correlation*, CRC, New York, US, 1995, 79-120.
16. S. J. Farr, I. W. Kellaway, D. Parry-Jones, and S. G. Woolfrey. Technetium as a marker of liposomal deposition and clearance in the human lung. *Intern. Symp. Control. Rel. Bioact. Mater.* **12**:219-220 (1985).
17. J. Becker and R.-J. Grassa. Suppression of phagocytosis by dexamethasone in macrophage culture: Inability of arachidonic acid, indomethacin, and nordihydroaiaretic acid to reverse the inhibitory response mediated by a steroid-inducible factor. *Int. J. Immunopharmac.* **7**:839-847 (1985).