Effect of Size and Lipid Composition on the Pharmacokinetics of Intravitreal Liposomes

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We investigated the influence of size and lipid composition on the pharmacokinetic behavior of liposomes and their contents in the rabbit eye. Small and large unilamellar vesicles (SUV and LUV), prepared with and without cholesterol in the membrane, were injected intravitreally in rabbits. The vesicles were labelled with 125I and contained 51Cr-EDTA in the aqueous compartment. The mode of elimination of the vesicles from the vitreous humor is uncertain but may be via the anterior route; 51Cr-EDTA, like gentamicin, probably is eliminated by the anterior route. The rate of clearance of the lipid label appeared to be related to the size but not to the cholesterol content of the liposomes. Liposome-encapsulation prolonged the half-life of 51Cr-EDTA by up to 11-fold in the vitreous humor of normal eyes. The prolongation was greatest with cholesterol-containing vesicles, presumably because these are most stable, and was somewhat greater with large than with small vesicles. For SUV and LUV, the rate of elimination of 51Cr-EDTA from the normal eye was determined mainly by the rate of leakage from the liposomes, whereas for SUV-cholesterol and LUV-cholesterol, it was determined mainly by the rate of clearance of the liposomes themselves. Both 51Cr-EDTA and liposomes (125I label) had a shorter half-life in infected than in normal eyes. Encapsulation of 51Cr-EDTA prolonged its half-life by up to sevenfold in infected eyes; the effect was greatest with cholesterol-containing vesicles. These results suggest that both the structure of the liposome and the state of the eye may markedly affect the pharmacokinetic behavior of intravitreal liposomes. Invest Ophthalmol Vis Sci 28:893–900, 1987

There has been increasing interest in the use of liposomes as a vehicle for the delivery of drugs. These preparations have been given subconjunctivally,1 topically,2,3 and intravitreally4,5 to experimental animals in ophthalmologic studies. Because of the comparatively large size of liposomes, the encapsulated drugs are retained in the circulation or at sites of injection for relatively long periods. This retentive effect may offer pharmacokinetic advantages1 or, in the case of agents that produce acute toxicity, may have toxicologic advantages.4,5

Water-soluble drugs such as penicillins, cephalosporins, and aminoglycosides are captured in the aqueous phase of liposomes. The drugs escape either by leakage through the membranes of intact liposomes or by diffusion from degraded or destabilized liposomes. Both modes of escape contribute to the half-life of the drug in the compartment of interest. The physicochemical properties of liposomes determine their rate of drug leakage and their rate of natural degradation. Inclusion of cholesterol in liposomes seems to increase their resistance to biodegradation.6

The purpose of the present study was to examine the impact of the size and composition of liposomes on the pharmacokinetic behavior of the vesicles and their encapsulated contents in the vitreous humor. For this purpose, we used 125I-labeled BPE liposomes (lipid label) containing 51Cr-EDTA. The latter was chosen as a convenient marker for an aqueous-phase agent; its half-life in the vitreous humor of the rabbit eye was found to be similar to that of gentamicin.

Materials and Methods

Materials

Phospholipids were obtained from Avanti Polar Lipids (Birmingham, AL). Cholesterol and tocopherol acid succinate were products of Sigma Chemical Co. (St. Louis, MO). Chromium-51-ethylenediaminetetraacetic acid complex (51Cr-EDTA) and 125I were products of New England Nuclear (Boston, MA). The 51Cr-EDTA was purified before use on a Bio Gel P-2 (BioRad; Richmond, CA) 1 × 30 cm column, eluted with phosphate buffered saline (PBS, 137 mM NaCl, 2.6 mM KCl, 6.4 mM Na2HPO4; pH, 7.4) at a flow rate of 0.7 ml per minute. Fractions of 0.7 ml were collected and the material eluting in fractions 20–26,
Table 1. Characteristics of liposomes for intravitreal injection

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Lipid content* (molar ratio)</th>
<th>Lipid concentration (μmol/ml)</th>
<th>Mean diameter of vesicles (μm)</th>
<th>Polydispersity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small unilamellar vesicles (SUV)</td>
<td>EPC/EPG/TS (9/1/0.05)</td>
<td>25</td>
<td>0.06</td>
<td>1.1</td>
</tr>
<tr>
<td>Small unilamellar vesicles with cholesterol (SUV-chol)</td>
<td>EPC/EPG/chol/TS (9/1/8/0.05)</td>
<td>25</td>
<td>0.06</td>
<td>1.1</td>
</tr>
<tr>
<td>Large unilamellar vesicles (LUV)</td>
<td>EPC/EPG/TS (9/1/0.05)</td>
<td>25</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Large unilamellar vesicles with cholesterol (LUV-chol)</td>
<td>EPC/EPG/chol/TS (9/1/8/0.05)</td>
<td>25</td>
<td>0.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* EPC = egg phosphatidylcholine; EPG = egg phosphatidylglycerol; TS = tocopherol succinate; and chol = cholesterol.

which is the $^{51}$Cr-EDTA, was encapsulated in liposomes. The lipid label, p-hydroxybenzaminedihexadecylphosphatidylethanolamine ($^{125}$I-BPE), was prepared and iodinated as described previously, and trace amounts were incorporated into the liposomes during their formation. All other chemicals were reagent grade or better.

**Preparation of Liposomes**

The lipids were deposited on the sides of a sterile round bottom flask by evaporation from a chloroform solution. Sterile PBS containing $^{51}$Cr-EDTA was added to the flask to hydrate the lipids yielding a final lipid concentration of 50 μmol/ml. Small unilamellar vesicles (SUV) were made by sonication at room temperature for 1 hr in a bath-type sonicator (Lab Supplies; Hicksville, NY). Large unilamellar vesicles (LUV) were prepared by the reverse phase evaporation method. Nonencapsulated $^{51}$Cr-EDTA was removed from the liposomes by placing them in sterile dialysis tubing (Spectrum Medical Industries; Los Angeles, CA) and dialysing them against 200 volumes of sterile PBS changed two times over a 24 hr period. The LUV were transferred aseptically to sterile tubes, diluted with sterile PBS to 25 μmol lipid/ml and stored under nitrogen at 4°C until use. The sterility of all preparations was verified by subculture on agar just before each use.

The particle diameter of each preparation was measured by dynamic light scattering using a Malvern light scattering monochromator and autocorrelator coupled to a Spectrophysics 15-mW helium (633 nm) fixed wavelength laser. The lipid composition, particle diameter and polydispersity index of each preparation is summarized in Table 1. The polydispersity index is a measure of the heterogeneity of the particle dispersion and is the ratio of the weight average diameter to the number average diameter of the preparation. A value of 1 indicates a monodisperse preparation. The lipid concentration was determined by measurement of the lipid phosphate using the Bartlett assay. The initial radioactivity for the four preparations ranged from $1.01 \times 10^6$ to $1.61 \times 10^6$ cpm/ml for $^{125}$I and $3.13 \times 10^4$ to $2.12 \times 10^5$ cpm/ml for $^{51}$Cr.

**Determination of Leakage of $^{51}$Cr-EDTA From Liposomes**

The rate of leakage of $^{51}$Cr-EDTA from liposomes was determined by dialysis into PBS as previously described. Aliquots of liposomes containing 1–2 × 10⁵ cpm of $^{51}$Cr-EDTA in 1 ml of PBS were placed in 10 mm cellulose dialysis tubing (Spectrum Medical Industries; Los Angeles, CA). The tubes were flushed with nitrogen, sealed at both ends and placed in 10 ml of PBS in conical centrifuge tubes. At timed intervals the bags were transferred to new tubes containing PBS and the total radioactivity that had leaked through the tubing into the reservoir was measured. Under these conditions 85% of nonencapsulated $^{51}$Cr-EDTA diffused out of the dialysis tubing in 1 hr. The rate of leakage was determined at 4°C and 37°C. To examine the effect of a biologic fluid, the PBS inside the dialysis tubing was replaced by fetal calf serum so that the final serum concentration was 80%; the rate of leakage of the $^{51}$Cr-EDTA was determined at 37°C.

**Intravitreal Injections**

Pigmented rabbits weighing 1.5–2.5 kg were used. All studies were carried out in accordance with the ARVO Resolution on the Use of Animals in Research. The animals were tranquilized by an intramuscular injection of ketamine (44 mg/kg) and acepromazine (0.25 mg/kg). Intravitreal injections of liposomes or of $^{51}$Cr were given in the superior quadrant of the eye, about 6 mm behind the limbus. One hundred microliters of the liposomal suspension were injected slowly...
into the center of the vitreous humor from a plastic tuberculin syringe through a 27-gauge needle. Because preliminary studies (unpublished data) showed no detectable radioactivity in the blood or in the contralateral eye following intravitreal injection of these liposome preparations, we assumed that there would be no significant crossover between the eyes of a pair and, therefore, used both eyes of each rabbit as independent samples.

Rabbits were killed at varying intervals after intravitreal injection and the eyes were promptly enucleated. The aqueous humor was aspirated, the cornea and iris were excised, the lens was removed and the vitreous humor was expressed carefully from the globe onto weighing paper, with care not to avulse the retina. Essentially all of the vitreous humor usually was extruded in the procedure; if any was seen clinging to the retina, it was gently separated and added to the larger sample. The retina, choroid, and sclera were gently dissected apart. A sample of venous blood also was obtained just before death of the animal.

Additional animals were studied after the induction of bacterial endophthalmitis by bilateral intravitreal injection of 500 colony-forming units of Staphylococcus aureus 209P. The liposomes were injected after the red reflex was lost, which occurred 24–48 hr after infection.

In general, four to six eyes were studied with each preparation at each interval in normal or infected eyes. In certain instances, as few as two or as many as eight eyes were studied.

**Assay Procedures**

All samples were counted on a gamma scintillation spectrometer. The activity of $^{125}$I was measured at 10–80 keV and that of $^{51}$Cr was measured at 170–760 keV. The vitreous humor was counted together with the weighing paper on which it was extruded; preliminary studies showed that about 5% of the counts were retained on the paper. To correct for spillover from the $^{51}$Cr channel to the $^{125}$I channel for samples in which the measured counts per minute (cpm) from the $^{51}$Cr channel were more than 20% of the cpm from the $^{125}$I channel, we used the following procedure:

Corrected $^{125}$I cpm = cpm in $^{125}$I channel minus efficiency factor of $^{51}$Cr in $^{125}$I channel × cpm in $^{51}$Cr channel.

Corrected $^{51}$Cr cpm = cpm in $^{51}$Cr channel minus efficiency factor of $^{125}$I in $^{51}$Cr channel × corrected $^{125}$I cpm.

This approach slightly overestimates the counts in the $^{51}$Cr channel and slightly underestimates those in the $^{125}$I channel. For samples in which the cpm in the $^{51}$Cr channel were less than 20% of the cpm in the $^{125}$I channel, the reciprocal procedure was used, correcting first for $^{51}$Cr. This slightly overestimates counts in the $^{125}$I channel and slightly underestimates those in the $^{51}$Cr channel. All values were corrected for decay and normalized to the time of injection. The coefficient of variation of the mean of the radioactivity remaining in the vitreous humor at each time point was between 10–40% for the nonencapsulated $^{51}$Cr-EDTA, 8–55% for the encapsulated $^{51}$Cr-EDTA and 10–55% for the $^{125}$I-BPE.

**Calculation of Half-Lives**

The Fit Line routine of the Prophet system (Biotechnology Resources Program, National Institutes of Health) was used to perform a regression analysis of the log of the concentration data to determine the half-lives for clearance of the nonencapsulated $^{51}$Cr-EDTA and the $^{125}$I-BPE. The data were fit to the slope of observed radioactivity remaining in the eye at each time point; we did not assume that 100% of the intended dose was present at 0 time. Single exponential clearance and equal variance at each time point were assumed in the analysis of the data. A covariance analysis of the slopes of the regression lines was performed using the Test Lines routine. The slopes of the regression lines for the $^{125}$I-BPE and the $^{51}$Cr-EDTA were compared within each group: SUV versus LUV; cholesterol containing versus noncholesterol containing liposomes. An $\alpha$ value of 0.05 was considered significant.

A two-compartment model was assumed to describe the elimination of encapsulated $^{51}$Cr-EDTA from the eye (Fig. 1). One compartment was assumed to be the liposome that underwent elimination from the vitreous humor at a rate that was described by the elimination of the $^{125}$I-BPE. A second compartment was the vitreous humor. $^{51}$Cr-EDTA leaks from the liposome into this compartment. The released $^{51}$Cr-EDTA was assumed to leave the eye at the same rate as the free $^{51}$Cr-

![Fig. 1. Two-compartment model describing elimination of $^{51}$Cr-EDTA from the eye following injection encapsulated in liposomes. $K_{10}$ represents the elimination rate constant of the liposomes from the eye. $K_{12}$ represents the leakage of $^{51}$Cr-EDTA from the liposome into the eye. $K_{20}$ represents the elimination rate constant of the free $^{51}$Cr-EDTA from the eye.](image-url)
the free $^{51}$ Cr-EDTA from the vitreous humor.

and $t =$ the half-time for disappearance of total $^{51}$ Cr-

model to fit the leakage of the $^{51}$ Cr-EDTA from the liposome; and $K_{20} =$ the first order rate constant for elimination of liposomes from the eye; $K_{12} =$ the first-order rate constant for leakage of the $^{51}$ Cr-EDTA from the liposome; and $K_{20} =$ the first order rate constant for elimination of the free $^{51}$ Cr-EDTA from the vitreous humor.

The equation was solved for $K_{12}$ by setting $F_t = 0.5$ and $t =$ the half-time for disappearance of total $^{51}$ Cr-EDTA from the eye when encapsulated in liposomes. This "t" was determined using the DRUGFUN routine of Prophet for a two compartment model to fit the elimination data generated by encapsulating $^{51}$Cr-EDTA in liposomes. This approach assumes that the rate constants were first order, and there is no back flux of any of the components.

**Results**

Following intravitreal injection of $^{125}$I-BPE labelled liposomes containing $^{51}$Cr-EDTA into normal eyes, the majority of both labels was found in the vitreous humor (Table 2). The only other ocular sites that contained more than 1% of the injected dose of either label were the lens, sclera, choroid, and retina. There were no marked differences in distribution among the various liposome-preparations or between the results obtained 1 and 72 hr after injection; therefore, the results shown in Table 2 are for all four liposomal preparations at both time intervals. Nevertheless, at the low levels of radioactivity detected in extravitreal sites it is difficult to discern what is true uptake as opposed to contamination of the specimens by small amounts of adherent vitreous humor.

More than 85% of the injected dose of SUV or SUV-containing cholesterol (SUV-chol) was detectable in the vitreous humor 1 hr after injection. In contrast, lower values were found for LUV, LUV-containing cholesterol (LUV-chol) and $^{51}$Cr-EDTA. An additional 8–15% of each label could be found in extravitreal sites as early as one hour after injection with each preparation. Whereas the total recovery was nearly 100% for SUV and SUV-chol, some label could not be accounted for with the other preparations. The conjunctiva was swabbed briefly immediately after the injection to detect leakage from the injection site; however, this procedure never retrieved more than 1% of the injected dose. Moreover, the liposomes did not adhere to the plastic tuberculin syringe. It seems likely that either some eyes received slightly less than the intended dose or that some of the dose leaked from the eye without being detected. Why the extent of the phenomenon should differ among the various preparations is unclear. $^{125}$I-BPE has been shown previously to be a nonexchangeable marker for the lipid bilayer of the liposome.7 The slope of its elimination curve from the vitreous humor was used to calculate the half-life of the various liposome preparations in the vitreous humor. An example is given for SUV-chol in Figure 2. Overall, the half-life of the $^{125}$I-BPE label ranged from 9–20.4 days, being shortest for the two SUV preparations and longest for the two large types of vesicles ($P < 0.05$) (Table 3). This suggests that the diameter of the liposomes was a determinant of their rate of elimination from the vitreous humor.

Injected by itself, $^{51}$Cr-EDTA had a half-life of only

<table>
<thead>
<tr>
<th>Liposome type*</th>
<th>$^{51}$Cr-EDTA†</th>
<th>No. of eyes</th>
<th>$^{125}$I lipid*</th>
<th>No. of eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV</td>
<td>3.9 ± 1.1</td>
<td>12</td>
<td>9.0 ± 1.5</td>
<td>20</td>
</tr>
<tr>
<td>SUV-chol</td>
<td>7.6 ± 1.8</td>
<td>15</td>
<td>9.9 ± 1.5</td>
<td>27</td>
</tr>
<tr>
<td>LUV</td>
<td>7.1 ± 0.5</td>
<td>30</td>
<td>20.4 ± 3.6</td>
<td>30</td>
</tr>
<tr>
<td>LUV-chol</td>
<td>12.5 ± 1.0</td>
<td>39</td>
<td>20.1 ± 2.4</td>
<td>39</td>
</tr>
<tr>
<td>Nonencapsulated</td>
<td>1.1 ± 0.1</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* SUV = small unilamellar vesicles; SUV-chol = small unilamellar vesicles with cholesterol; LUV = large unilamellar vesicles; and LUV-chol = large unilamellar vesicles with cholesterol.
† Values are the mean and SE of the mean determined as described in the Materials and Methods section.
‡ $^{51}$Cr-EDTA injected in PBS.
Table 4. Rate of leakage of $^{51}$Cr-EDTA from liposomes in vitro and in vivo

<table>
<thead>
<tr>
<th>Liposome type*</th>
<th>PBS/4°C</th>
<th>PBS/37°C</th>
<th>80% serum/37°C</th>
<th>Vitreous humor (normal eyes)†</th>
<th>Vitreous humor (infected eyes)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>SUV-chol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>24</td>
<td>5.4</td>
</tr>
<tr>
<td>LUV</td>
<td>&gt;180</td>
<td>14</td>
<td>0.21</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td>LUV-chol</td>
<td>&gt;180</td>
<td>81</td>
<td>2</td>
<td>29</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* SUV = small unilamellar vesicles; SUV-chol = small unilamellar vesicles with cholesterol; LUV = large unilamellar vesicles; and LUV-chol = large unilamellar vesicles with cholesterol.
† See text for method of calculation.

1.1 days. Incorporation into liposomes prolonged the half-life of $^{51}$Cr-EDTA in the vitreous humor by up to 11-fold. The effect was significant with each of the four preparations when compared with the nonencapsulated $^{51}$Cr-EDTA ($P < 0.05$). For each diameter of vesicle, the cholesterol-containing formulation caused a greater extension of the half-life of $^{51}$Cr-EDTA than the non-cholesterol containing one ($P < 0.05$, Table 2). Indeed, although the $^{125}$I-BPE label remained in the eye longer with LUV than with SUV liposomes, the intraocular half-life of $^{51}$Cr-EDTA was more effectively prolonged by adding cholesterol to the formulation than by increasing the liposome diameter. These data are consistent with many other studies that show that cholesterol stabilizes the liposome membrane to the disrupting effects of biologic fluids.

The latter finding was also consistent with the in vitro stability of the liposomes used in this study. The half-life for leakage of $^{51}$Cr-EDTA from the LUV and LUV-chol was greater than 6 months for liposomes stored at 4°C in PBS and was 14 days and 81 days, respectively, for liposomes stored at 37°C in PBS. Much shorter half-life values were obtained when the liposomes were stored at 37°C in 80% fetal calf serum (Table 4).

Table 5. Half-life of liposome-associated radiolabels following intravitreal injection into infected rabbit eyes

<table>
<thead>
<tr>
<th>Liposome type*</th>
<th>$^{51}$Cr-EDTA†</th>
<th>No. of eyes</th>
<th>$^{125}$I lipid†</th>
<th>No. of eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV</td>
<td>1.5 ± 1.0</td>
<td>10</td>
<td>7.8 ± 1.0</td>
<td>10</td>
</tr>
<tr>
<td>SUV-chol</td>
<td>3.6 ± 0.5</td>
<td>10</td>
<td>7.4 ± 0.6</td>
<td>18</td>
</tr>
<tr>
<td>LUV</td>
<td>1.2 ± 0.5</td>
<td>11</td>
<td>4.7 ± 0.4</td>
<td>11</td>
</tr>
<tr>
<td>LUV-chol</td>
<td>3.3 ± 0.3</td>
<td>14</td>
<td>6.8 ± 0.3</td>
<td>14</td>
</tr>
<tr>
<td>Nonencapsulated</td>
<td>0.5 ± 0.1</td>
<td></td>
<td></td>
<td>16</td>
</tr>
</tbody>
</table>

* SUV = small unilamellar vesicles; SUV-chol = small unilamellar vesicles with cholesterol; LUV = large unilamellar vesicles; and LUV-chol = large unilamellar vesicles with cholesterol.
† Values are the mean and SE of the mean determined as described in the Materials and Methods section.
‡ $^{51}$Cr-EDTA injected in PBS.

Because of the gelatinous consistency of the vitreous humor, it was not possible to measure the rate of leakage of $^{51}$Cr-EDTA from liposomes in the vitreous hu-
To approach the question of the rate of leakage from liposomes in vivo, a two-compartment model was used in which the central compartment was the liposome (Fig. 1). The rate constant of the elimination of liposomes (125I label), nonencapsulated 51Cr-EDTA and encapsulated 51Cr-EDTA are known (Table 3). The general equation for the two-compartment model could then be solved to determine the rate of leakage of 51Cr-EDTA from the liposomes in the vitreous humor. The calculated half-life for leakage from SUV was 4 days and from LUV was 8 days; much larger values were calculated for the cholesterol-containing preparations (Table 4). The half-life for leakage of liposomes in the vitreous humor was much longer than the corresponding values in serum in vitro. Thus, the destabilizing factors in the vitreous humor are not as potent as those found in serum, and cholesterol has a stabilizing effect in both instances.

To ensure that the prolongation of the intravitreal half-life of 51Cr-EDTA was because of its delayed release from liposomes rather than some other effect of liposomes on the egress of 51Cr-EDTA from the eye, the authors injected 51Cr-EDTA plus "empty" liposomes (LUV-chol) into normal rabbit eyes. Eyes were sampled 1, 24, 72, and 168 hr after injection. The mean half-life was 1.2 ± 0.1 days, which is not significantly different from the half-life of 51Cr-EDTA without liposomes (1.1 ± 0.1 days).

To examine the effect of inflammation on the rate of clearance of liposomes a model bacterial infection was used. Table 5 shows the half-life for clearance of the two labels from infected eyes. The distribution of 125I-BPE and 51Cr-EDTA in extravitreal sites of infected eyes (data not shown) was similar to that shown for normal eyes (Table 3). The percentage of the injected dose of radioactivity in the vitreous humor 1 hr after injection was generally in the 50–70% range for each preparation, presumably for the same reasons as were discussed with respect to normal eyes. The half-life of the 125I-BPE was shorter in infected eyes than in normal eyes for each liposome preparation; however, the effect was particularly striking for the LUV and LUV-chol. The half-life of nonencapsulated 51Cr-EDTA in infected eyes was also significantly shorter than in normal eyes (0.5 versus 1.1 days, P < 0.01) but was prolonged when the 51Cr-EDTA was incorporated into liposomes (Table 5). The prolongation ranged from twofold to threefold for the noncholesterol-containing liposomes and sixfold to sevenfold for the cholesterol-containing liposomes. The effect was only significant for the two cholesterol containing preparations (P < 0.01). The two-compartment model was used to calculate the half-life for leakage of the 51Cr-EDTA from liposomes in the infected eyes. The half-life for leakage of 51Cr-EDTA from SUV and LUV was less than one day but was much longer for SUV-chol and LUV-chol (Table 4).

**Discussion**

The objective of this study was to examine the effect of the size and composition of liposomes on their pharmacokinetic disposition following intravitreal injection. 51Cr-EDTA was used as an aqueous-phase marker. 51Cr-EDTA has a molecular weight (362.2) that is somewhat less than that of gentamicin (463); its half-life in the vitreous humor of normal and infected rabbit eyes (1.1 days and 0.5 days, respectively) is similar to that of gentamicin (1.0–1.3 days and 0.4–0.8 days, respectively). This suggests that 51Cr-EDTA, like gentamicin, is eliminated by the "anterior route" (ie, by diffusion to the anterior chamber and through the canal of Schlemm). However, we cannot be certain that the 51Cr-EDTA is eliminated via the anterior route because we did not carry out detailed measurements of radioactivity in the anterior segment.

Our concept is that, following the injection of liposomes into the vitreous humor, several processes begin simultaneously: (1) intact as well as degraded vesicles begin to be cleared from the vitreous humor; (2) the vesicles start to leak their contents into the vitreous humor; and (3) the contents of the liposomes begin to be eliminated—in the case of 51Cr-EDTA, by the anterior route (Fig. 1). If one considers the 125I lipid label to be a marker for the liposomal membrane, the slow rate of clearance of the label compared with that of encapsulated 51Cr-EDTA (Table 3) suggests that most of the 51Cr-EDTA had leaked out before the liposomes left the vitreous humor. This would indicate that the liposomes acted as a slow-release device for the 51Cr-EDTA just as they do for liposome-encapsulated gentamicin injected subconjunctivally.

The liposomes themselves could be eliminated by diffusion via the anterior route or through phagocytosis (eg, by pigment epithelial cells) or through degradation in situ, as well as by other mechanisms or a combination of processes. These data do not permit estimation of the relative contribution of these possible modes of elimination or whether the 125I label was cleared on intact vesicles or on degraded fragments. Only about 1–2% of the injected label was found in the choroid and retina (Table 2), which militates against an important contribution for phagocytic cells in these locations; however, more detailed mass balance studies would be needed to resolve this point. Although less than 1% of the dose of 125I was found in the anterior chamber even 72 hr after intravitreal injection, which
could argue against a major role for the anterior route, the rate of movement of the liposomes through the comparatively viscous vitreous humor could be much slower than the rate of egress from the anterior chamber. An anterior route of egress would fit the observation that SUV (presumably more mobile than LUV) had a shorter vitreal half-life than LUV. Given the available data, we favor an anterior route of elimination of liposomes from the vitreous humor.

The vitreal half-life of $^{125}$I after injection into normal eyes was longer when the label was present on large rather than small vesicles (20 versus 9–10 days) but was unaffected by the cholesterol content of the liposomes (Table 3). Therefore, size but not lipid composition was the important determinant of the rate of clearance of liposomes from uninfected eyes.

The half-life of $^{51}$Cr-EDTA in the vitreous humor of normal eyes was prolonged by 3.5- to 11-fold by liposome-encapsulation. With both sizes of vesicle, the increase in half-life was greatest for the cholesterol-containing formulation. This is in agreement with previous reports showing that cholesterol stabilizes liposomal membranes. The vitreal half-life of $^{51}$Cr-EDTA was longest when the marker was encapsulated in LUV-chol, presumably because two effects were operating (ie, the larger size of the vesicles reduced their rate of clearance and the cholesterol reduced their "leakiness"). Therefore, both the size and the lipid composition of the liposomes affected the half-life of $^{51}$Cr-EDTA. Once the $^{51}$Cr-EDTA was extraliposomal, its vitreal half-life was the same as in eyes without liposomes, as shown by experiments in which $^{51}$Cr-EDTA was injected with "empty" liposomes.

The half-life of $^{125}$I was shorter in infected eyes (4.7–7.8 days) than in normal eyes (9.0–20.4 days) especially for LUV and LUV-chol. The reasons for the higher rate of clearance of liposomes (lipid label) in infected eyes are unknown but could include an enhanced rate of diffusion of liposome through the more liquid vitreous humor in infection, an increased rate of flow from the vitreous humor to the anterior chamber, an augmented uptake by fixed or mobile phagocytes, or a breakdown in some of the blood–ocular barriers. Why large liposomes should have been affected more than small ones is not apparent. The half-life of the $^{51}$Cr-EDTA, like that of gentamicin, was significantly shortened in infected eyes, again for obscure reasons. The net effect was that encapsulation of $^{51}$Cr-EDTA prolonged its half-life in the vitreous humor of infected eyes by twofold to sevenfold, a range slightly lower than that in normal eyes. For both sizes of vesicle the prolongation was greatest with cholesterol-containing preparations.

Comparison of the data of Tables 3 and 4 suggests that for SUV and LUV the rate of egress of $^{51}$Cr-EDTA was determined mainly by the rate of leakage from the liposomes, whereas for SUV-chol and LUV-chol it was determined mainly by the rate of elimination of the liposomes themselves. This fits with the concept that cholesterol stabilizes liposomes so that, for cholesterol-containing liposomes, leakage of $^{51}$Cr-EDTA is too slow to be rate-limiting and clearance of the liposomes themselves becomes the determinant of the rate of clearance of $^{51}$Cr-EDTA. A stabilizing effect of cholesterol has been documented for liposomes in the presence of serum and has now been demonstrated in the biologic fluids of the eye. In the infected eye, the rate of leakage of $^{51}$Cr-EDTA from liposomes was much faster than in the normal eye (Table 4) and seemed to be the major contributor to the half-life of encapsulated $^{51}$Cr-EDTA in all four preparations. The faster rate of leakage in infected eyes than normal eyes may reflect a disruptive effect of inflammatory products on the liposomes.

These results substantiate the anticipated beneficial effects of liposomes in prolonging the half-life of a water-soluble agent in the vitreous humor. In addition, they show that the size and composition of the liposomes and the state of the eye (ie, normal or infected), have pronounced effects on the disposition of liposomes and their contents after intravitreal injection. These considerations will certainly be important in the design of liposomal formulations of therapeutic agents for intravitreal administration.

Key words: liposome, cholesterol, intravitreal, pharmacokinetics, infection

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