A Potential Method For Local Drug and Dye Delivery in the Ocular Vasculature

Ron C. Zeimer,* Bahram Khoobehi,*^ Michael R. Niesman,† and Richard L. Magin†

We propose a new drug and dye delivery system that would allow repeated release of substances in the ocular vasculature by an externally controlled mechanism. The substances are encapsulated in heat-sensitive liposomes, which are lysed by locally applying a heat pulse produced by an argon laser. The system was tested by investigating the release of carboxyfluorescein encapsulated in the liposomes. The liposome suspension was incubated at 37° or 38.5°C and irradiated at different powers and pulse durations. The amount of dye released was monitored by fluorophotometry and compared with the concentration obtained when the liposomes were lysed at their transition temperature of 41°C. The results showed that 85% of the encapsulated substance can be released. Moreover, a dramatic contrast was observed between the fluorescence before and after the lysis. Presently, the energy density is higher than but close to the maximal permissible exposure for humans. The release mechanism with the short laser pulse appeared to be similar to that present when liposomes were heated slowly. Invest Ophthalmol Vis Sci 29:1179-1183, 1988

At present, drug and dye tracers are delivered to the eye mainly through topical and systemic routes. Topical delivery cannot achieve high intraocular concentrations of many agents because of the limited penetration through the cornea or sclera. The systemic route is limited by the fact that the whole body is exposed to the drug, thereby restricting the permissible dose. Therefore, for drugs, it would be advantageous if the substance could be delivered directly to a specific site in the eye. In this way, a high local concentration could be obtained at the desired site, thus enhancing the therapeutic effect and lessening the side effects at other sites. Regarding the delivery of dyes, it would be advantageous if one could repeatedly obtain a bolus of dye at a specific site in the ocular vascular system. Presently, by injecting the dye systemically, one can obtain a bolus, but it is ill-defined and the procedure cannot be repeated because the amount of drug already injected causes a significant background.

The above goals may be achieved by a new delivery system, whereby the active substance is encapsulated in lipid vesicles, which are injected systemically and lysed at a controlled time and site by an external irradiating agent.

Vesicles made of synthetic phospholipids, called liposomes, can be used for this purpose to encapsulate active substances within their inner aqueous phase and to isolate the drug from the body fluids, thereby reducing toxic effects on other organs. We propose to exploit temperature-sensitive liposomes and release their contents at the appropriate site by heating them locally. Such liposomes prepared from dipalmitoylphosphatidylglycerol (DPPG) and dipalmitoylphosphatidylcholine (DPPC) exhibit, in serum, a dramatic and fast increase in release at a phase transition, which occurs around 41°C. Therefore, with a rise of only 4°C above body temperature, one could trigger locally the release of the liposomes’ content. We propose to achieve this temperature elevation by the use of electromagnetic radiation. In brief, liposomes will be injected into body fluids such as blood or vitreous, and a pulse of light will be delivered to the target site. The light absorbed by the tissue will heat the liposomes, causing the local release of the drug or dye at a high concentration. The procedure can be repeated as long as the liposomes are present in the fluid.

In this article we will present the results of preliminary in vitro studies that show the feasibility of this new method. To test and quantify the lysis mecha-
nism, we used the fluorescent dye carboxyfluorescein (CF) and monitored its release by fluorophotometry.

Materials and Methods

Preparation of Liposomes

The lipids, DPPC and DPPG (Avanti Polar Lipids, Birmingham, AL), were used without further purification. The fluorescent dye, 6-CF (Molecular Probes, Junction City, OR) was repurified by a method described by Ralston and coworkers. An ethanol solution of CF was boiled with decolorizing carbon followed by recrystallization of the dye in ethanol water. After this procedure, a 250 mM solution of the dye in water was obtained by the addition of sufficient 1 M NaOH to raise the pH to 7.4. The solution was eluted with distilled water through a 25 by 1.6 cm column (Sephadex LH-20). The purified dye was diluted with water to make it isosmotic (290 mOsm); this resulted in a concentration of approximately 100 mM.

Large unilamellar vesicles were prepared using the reverse-phase evaporation method of Szoka and Papahadjopoulos with slight modifications. Briefly, liposomes were made using 125 mg of a 4-to-1 mixture by weight of (DPPC and DPPG). The aqueous phase consisted of the above-mentioned 100 mM solution of repurified CF at pH 7.4. The lipids were dissolved in an organic phase and combined with 4 ml of the aqueous phase. After sonication to form an emulsion, the mixture was placed in a rotary evaporator. The pressure was lowered and the organic phase was removed at 50°C. The liposome suspension was brought to approximately 5 ml by adding buffer, allowed to cool, and dialyzed against HEPES buffered saline (145 mM NaCl, 2 mM KCl and 10 mM HEPES) to remove unencapsulated dye.

Laser Delivery

For the experiment the liposome preparation was diluted 400 times in calf serum. This dilution coefficient was chosen to match the one that would be achieved following slow injection of about 12 cc of liposomes in humans. The preparation was placed in a cuvette consisting of two flat glass plates separated by 0.8 mm and incubated in a bath at 37° or 38.5°C. A commercial ophthalmic argon laser system (Coherent Radiation, Palo Alto, CA) was used to deliver the laser beam in the blue-only mode. The cuvette was displaced from the focal plane so that the beam diameter would be 2 mm and thereby cover the whole sample.

The amount of CF released was assessed by measuring the fluorescence with a fluorophotometer (Coherent Radiation). The release yield was calculated from the ratio between the fluorescence (above initial background at 37°C) of the irradiated sample and that of a control sample heated at the liposome transition temperature of 41°C. The samples were diluted with phosphate buffer to keep the concentration in the linear range of the fluorophotometer.

Results

Release Yield versus Energy Density For Different Exposure Times

The release yield was measured at different energy densities and at three laser pulse durations (100, 200 and 500 msec), and the results are summarized in Figure 1. It is noted from the figure that, for each exposure time, the release increases with the energy density, and up to 80% of the originally encapsulated dye is released. As the pulse length is increased, higher energies are needed to obtain the same amount of dye release.

Release Yield versus Energy Density For Two Initial Temperatures

The release yield was measured at different energy densities and at three laser pulse durations (100, 200 and 500 msec), and the results are summarized in Figure 1. It is noted from the figure that, for each exposure time, the release increases with the energy density, and up to 80% of the originally encapsulated dye is released. As the pulse length is increased, higher energies are needed to obtain the same amount of dye release.
Release Yield versus Solution Temperature

To better interpret the results obtained with a laser pulse, we have plotted in Figure 3 the liposome release as a function of temperature for preparations of liposomes placed in a bath and slowly heated to different temperatures. These data were obtained from the work of Magin and Niesman. This figure illustrates that when the initial temperature is higher a smaller temperature increment is needed to obtain a given release. Moreover, the graph indicates that the maximal release is between 80 and 90%.

Effect of CF Release on the Fluorescence

With the aid of a fundus camera equipped for fluorescein angiography, a photograph (Fig. 4) was obtained after irradiating for 200 msec a small portion of a sample with the laser set at an energy density of 4 joules/cm². The photograph illustrates that while CF is encapsulated inside the liposome there is little fluorescence. This is due to fluorescence quenching at high CF concentrations. On the other hand, where the laser is applied CF is released and fluoresces strongly following its dilution in the serum. As mentioned above, the dilution of the liposomes realistically simulates the dilution occurring in the vascular bed and the capillary diameter of 250 μm is similar to that of large ocular blood vessels.

Discussion

The use of liposomes for drug delivery has been the subject of many studies and several commercial companies are developing clinical applications for this delivery system. However, at present, there are no methods capable of repeatedly delivering drugs or dyes inside the eye at specific locations and times. We propose a method based on the use of heat-sensitive liposomes, which are lysed at a given location and time by a pulse of radiation delivered externally.

In this study we encapsulated CF because it strongly absorbs blue light from an argon laser and it can be used as a tracer for the quantification of the release mechanism. Our results show that the encapsulated dye can be released from the liposomes by a pulse of blue light. The energy density required to release a given amount of CF was found to increase...
with the pulse duration. This observation is easily explained by the dissipation of heat. This phenomenon is related to the dimensions of the cuvette. In blood vessels the time constant will be different due to the site of the vessel and the presence of flow. A separate investigation of time dependence will be necessary for in vivo application. Although short pulses reduce the energy density, very short pulses would not necessarily be the most advantageous for the application of this system to the eye. Since the liposomes are flowing through the portion of the retinal vessel exposed to the laser, longer pulse durations will allow more liposomes to be lysed by the laser beam, causing more dye or drug to be released into the vascular system. An optimal exposure time will have to be identified. It is of some interest to compare the energy density used in this experiment to the maximal permissible exposure for humans. For pulses of 200 msec the energy density needed for a release of 85% was 3.6 J/cm². For an irradiated area more than 190 μm in diameter, the maximum permissible energy density on the retina is about 1.8 J/cm². This indicates that the presently used energy density is close to the permissible value, which is typically ten times below the threshold for damage. The energy density necessary in vivo may well be lower due to the strong absorption of energy by red blood cells, which will most likely be the main mechanism of heating.

The study of the release yield versus initial incubation temperature indicated that less energy is required at a temperature of 38.5°C than at 37°C. This is easily explained by the fact that less energy is required to heat the liposomes to 41°C from 38.5°C than from 37°C. It is very instructive to compare our experimental results with those of Magin and Niesman, who measured the release after slowly heating the incubation bath to different temperatures. The general impression obtained by comparing Figures 2 and 3 is that the behavior is similar for liposomes lysed by the laser pulse and by direct heating of the solution. In both cases there is a plateau at about 80% release, and any further increment in laser energy or temperature does not enhance the amount of dye released from the liposomes. This behavior indicates that all the dye has been released from the liposomes. The fact that the maximum release is 85% due to the leakage of 15% of the dye following the incubation at 37°C. The similarity between the two experiments indicates that the short heating time caused by the laser pulse does not preclude complete release for times as short as 100 msec. This seems to imply that at 41°C the release of the liposomes' content is due to complete lysis of the membrane rather than to the enhancement of its permeability.

The implication of CF release on the fluorescence of the sample is illustrated in Figure 4. The background fluorescence of the sample before irradiation was very low in contrast to the very high fluorescence observed immediately after the release of the liposomes' content. This high contrast is encouraging, especially considering the fact that it was obtained with a dilution factor that is attained in humans during fluorescein angiography.

This new delivery system may open new avenues in fluorescein angiography and drug delivery. A release of dye induced at a localized area of the vascular bed would create a well defined bolus of dye not presently available clinically. The advantages of this localized bolus would be to visualize the retinal vasculature without the background of the underlying choroidal circulation or adjacent vessel networks. This would enhance the quality of the angiogram and isolate individual areas of perfusion or leakage. In addition, a better defined dye-front may allow quantitative measurements of blood velocity to be performed. The capability of repeating the procedure may allow one to perform measurements before and after induced physiologic changes.

Finally, drugs could be administered in a localized area of the retina without exposing the whole body to that substance. An example of the potential use of such a delivery system could be the administration of high concentrations of drugs to treat branch artery or vein occlusions.

At this point one can only speculate on the potential toxicity of this delivery system. The lipids themselves are not toxic; neither is carboxyfluorescein. The potential toxicity of drugs revolves around organs such as the liver, lungs and bone marrow which are involved in the clearance of liposomes and thus are exposed to the concentrated substances. This issue must be studied and its outcome will probably vary from drug to drug.

Key words: fluorescein, retina, drug delivery, liposomes

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