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OCULAR CONTROLLED DELIVERY OF BRIMONIDINE USING LIPOSOMES AS A PARTICULATE CARRIER

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ABSTRACT

The ocular activity of a single dose of brimonidine 0.2 % in solution and liposomal formulations was evaluated in 28 adult patients with recently diagnosed primary open angle glaucoma. Multilamellar liposomes were prepared from dipalmitoyl phosphatidcholine and cholesterol. Stearylamine and dicetyl phosphate were used to modify the net surface charge of liposomes. The evaluation of the performance of various formulations of the drug was assessed on the basis of the influence of the drug on the intraocular pressure. Results demonstrated that the intra-ocular activity of the drug using various liposomal formulations was greater than that of solution form. Moreover, concerning the liposomal surface charge, the drug activity was greatest for positively charged liposomes, less for neutral liposomes and least for negatively charged liposomes. This could be attributed to electrostatic attraction between the negatively charged surface of the corneal epithelial and positively charged liposomal surface. In conclusion, it was clearly observed that liposomal ophthalmic drug delivery system seems to be a promising approach for the selective targeting of the drug.

INTRODUCTION

As selective drug delivery system, liposomes have been and continue to be extensively used to improve the therapeutic index of known active drugs. Liposomes are vesicles composed of a lipid membrane enclosing an aqueous volume. Drug can be entrapped either in the lipid or the aqueous phase. Therefore, liposomal structures can encapsulate lipophilic drugs within the lipid bilayers and hydrophilic drugs in the aqueous space[1-4]. Thus liposomal lipid composition and the physicochemical properties of encapsulated drugs are factors that must be considered when designing liposomes as drug carriers[5-9].

The potential usefulness and advantages of liposomes as a novel ophthalmic drug delivery system include their abilities to control the rate of release of the encapsulated drug, to protect the drug from the metabolic enzymes present at the tear/corneal epithelium interface and their ability to form intimate contact with corneal and conjunctival surfaces, thereby increasing the possibility of ocular drug absorption[10-14].

All the currently approved treatments for glaucoma and also traditional antiglaucoma therapies have focused on the reduction or control of intraocular pressure. The use of antiglaucoma medications requires chronic administration of antiglaucoma drugs. Currently, the classes of drugs used in the management of glaucoma include topical beta-adrenergic-receptor antagonists, cholinergic agents, topical carbonic anhydrase inhibitors, prostaglandin analogues, nonselective adrenergic-receptor agonists, and selective alpha-adrenergic-receptor agonist[15].

Brimonidine is a highly selective alpha-adrenergic agonist indicated for the lowering of intraocular pressure in patients with glaucoma or ocular hypertension[16]. It is an effective ocular hypotensive regimen for a wide range of patients including those with early stage and more advanced glaucoma.

Moreover, brimonidine whether given as mono or adjunctive therapy, invariably appeared safe, even after four years of continuous use[17-19]. Also, since its introduction in 1996, use of brimonidine tartrate 0.2% ophthalmic solution (Alphagan) has become increasingly popular for the initial and long-term management of ocular hypertension[19].

The purpose of the present study was to evaluate and compare the activity of brimonidine in solution and various liposomal formulations on the basis of intraocular pressure lowering effect. The time course of the drug effect on the intraocular pressure was traced for different formulations after topical instillation into patients with bilaterally recently diagnosed primary open angle glaucoma.

EXPERIMENTAL

1. Materials

Brimonidine tartrate (Sigma Chemical Co., St – Louis, Mo., USA), mono and dibasic sodium phosphate, sodium metabisulphite and sodium chloride, were all of pharmaceutical grade. L-α-dipalmitoyl phosphatidyl choline, dicetyl phosphate, stearylamine, cholesterol were all obtained from Sigma Chemical Co.,St. Louis, Mo., USA. Polycarbonate membrane and membrane holders were obtained from Nucleopore corp.

2. Patients

The study included 28 patients with bilaterally recently diagnosed primary open angle glaucoma. Medical and ocular histories were taken. Slit-lamp examination, gonioscopy, ophthalmoscopy, as well as measurement of intraocular pressure with applanation tonometer were performed.

Exclusion criteria included history of previous antiglaucoma medications, intraocular surgery, laser trabeculoplasty, any ocular inflammation or infection, any condition preventing reliable applanation tonometry and patients with diabetes mellitus, hypertension, cardiac or respiratory disorders.

Consent was obtained from all patients before the study. The patients were classified into 4 groups (each

group involved 7 patients). Both eyes of each patient were used, where a single 50 µL (0.2%) dose of brimonidine solution or the drug encapsulated in liposomal formulations (medicated) was applied to the right eye, while the control one (non-medicated) was applied to the left eye.

All patients were instructed to report any ocular or systemic adverse events. Blood pressure and heart rates were followed up.

3-Method

A) Preparation of multilamellar liposomes;

Three different compositions were used to produce either a neutral, positive, or negative surface charge to liposomes:

i) Neutral liposomes were prepared from dipalmitoyl phosphatidylcholine and cholesterol in the molar ratios of 7:3.

ii) Positively charged liposomes were prepared from dipalmitoyl phosphatidyl- choline, cholesterol and stearylamine in the molar ratios of 7:2:1.

iii) Negatively charged liposomes were prepared from dipalmitoyl phosphatidyl- choline, cholesterol and dicetyl phosphate in the molar ratios of 7:2:1.

Briefly, multilamellar liposomes were prepared by the technique of Bangham *et al.*[20]. The lipids were dissolved in a minimal amount of chloroform in 100 ml round-bottom flask. Chloroform was completely removed on a rotary evaporator under vacuum at 25° C until a thin and smooth film was formed. The lipid film was hydrated with the appropriate amount of isotonic phosphate buffer solution (PH 6.8 ± 0.2) containing the drug as follows:

Brimonidine tartrate was dissolved in propylene glycol and incorporated into the aqueous compartment of liposomes with the isotonic phosphate buffer to give 0.2% final concentration of the drug. The suspension was shaken gently by hand for about 1 hour under nitrogen gas at 25°C.

The resulting multilamellar liposomes were adjusted with the same buffer to yield a final concentration of 60 µ mol lipid /ml aqueous phase.

B) Sequential extrusion of the liposomal preparations:

A homogenous liposomal preparations with controlled particle size distribution was obtained by sequential extrusion method[21].

The total lipid concentration was 60 µ mol /ml and the liposomes were diluted to 12 µ mol /ml in the same buffer prior to extrusion. The preparations were forced several times through polycarbonate membrane filters with 3.0, 2.0, 1.0 and 0.8 µm pores. The extrusion process was accomplished at a relatively low pressure (about 10 pounds/square inch) in 25 mm membrane holder.

C) Determination of surface charge of liposomal preparations :

The sign of the net charge at the liposmal surface was determined by electrophoretic mobility using Carl Zeiss cytopherometer[32].

D) Determination of encapsulation efficiency of brimonidine within liposomes :

The encapsulated drug (entrapped within liposomes) was separated from the unencapsulated drug (free or unentrapped) by centrifugation at 8,000 rpm for 15 minutes at 4°C. The supernatants, which contained the unencapsulated drug, were carfully decanted and the liposomes were resuspended gently in the same buffer used in liposomal preparations. This procedure was repeated to ensure complete removal of free drug. The supernatant from each process was collected and assayed for determination of free drug as it was described by Abu-Zaid, S.S.[23]. The encapsulation efficiency was calculated as follow:

$$\frac{\text{Amount of encapsulated}}{\text{Total amount of drug}} \times 100$$

E) Intraocular pressure measurments;

The drug solution or liposomal formulations were instilled into the right eye, while the non-medicated formulation (control) was instilled into the left eye. A single 50 uL dose (0.2% brimonidine) of each formulation was instilled directly into the corneal surface of the eye.

The intraocular pressures were measured using applanation tonometer. The measurements were made for each formulation at certain time intervals until returned to the basal line to determine the following parameters: maximum response, time of maximum response, duration of action and area above the curve.

F) Statistical analysis

Statistical analysis was performed as described by Schuman J. S.[24]. The area above the intraocular pressure/time curve values were calculated using the trapezoidal method[25].

RESULTS AND DISCUSSION

The influence of liposomal surface charge on the encapsulation of brimonidine was studied. Table I summarizes the encapsulation efficiency for various lipid composition of positively, negatively and neutral multilamellar liposomes. The results clearly showed that the encapsulation efficiency was greatest for negatively charged liposomes, less for positively charged and least for neutral liposomes. These results could be due to drug-lipid interaction with regard to their physicochemical properties.

The drug activity was evaluated by measuring the intraocular pressure lowering effect, where it was reported that 0.2% ophthalmic solution of the drug is a selective α_2-adrenergic agonist for the management of ocular hypertension[19].

Figure (1) shows the time course of the intraocular pressure after instillation of the different formulations of the drug. The data were statistically interpreted in terms of area above the intraocular pressure/time curve, duration of action, maximum response and time of maximum response which have been taken in

consideration as parameters of drug activity (Table II).

I) Area above intraocular pressure/time curve:

Statistical analysis of the data indicated that, the differences between the solution and liposomal formulations, with regard to the area above the curve, were very highly significant ($p<0.001$). Moreover, the differences between positively charged liposomes and negatively charged or neutral type were also very highly significant ($p<0.001$). Accordingly, positively charged liposomal formulation had the largest area above the curve as compared to other formulations.

II) Duration of drug action:

The data presented in table II clearly revealed that the duration of action could be prolonged to 31 hours for positively charged liposomes. Thus, multilamellar liposomes with positive surface charge displayed the most prolonged effect compared to other formulations.

Statistical analysis of the data, concerning the duration of action, demonstrated that the differences between solution and liposomal formulations were very highly significant ($p<0.001$). Also, the difference between positively charged liposome and negatively or neutral type was very highly significant ($p < 0.001$).

III) Maximum response:

Concerning the maximum response parameter, the results in Table II and Figure (2) showed that liposomal formulations provided greater values compared to the solution form. Moreover, the intensity of the drug action depends to a great extent on the liposomal surface charge.

Apparently, the intensity of drug action could be arranged in the following order: positively > neutral > negatively charged liposomes .

IV) Time of maximum response :

Table II shows that the time required to reach the maximum response was greatest for positively charged liposomes as compared to other formulations. Also, statistical analysis of the data revealed that the differences in the time of maximum response were highly significant ($p <0.001$) between solution and any one of the liposomal formulations (Figure 2). Moreover, the differences between positively charged and negatively or neutral liposomes were very highly significant ($P <0.001$).

Summarizing the above-mentioned results, it was clearly observed that liposomal encapsulation of the drug produced a greater influence on the parameters of drug action compared with the solution form.

Furthermore, on comparing the different formulation concerning the liposomal surface charge, this study revealed that liposomes with positive surface charge displayed the greatest enhancement in drug activity. Thus, the liposomal surface charge will influence the behavior of the encapsulated drug. It can be concluded that, positively charged liposomal preparation represented an optimal formulation for improving the ocular bioavailability of the drug. This influence can be explained on the consideration that

(1) The main components of liposomes were materials that are present as naturally occurring constituents in cell membranes as phospholipids and cholesterol, and therefore they are biocompatible, biodegradable and of good bioacceptability[24,27].

(2) Cholesterol incorporation into the phospholipid bilayers of liposomes strongly controlled drug release which, in turn, would help in providing higher drug loading at ocular tissues and also increasing the drug binding affinity for the corneal surface of the eye[10,12,28].

(3) The presence of a number of lamellae or concentric lipid bilayers in multilamellar liposomes could be responsible for the delay or prolongation of drug action, where they act as a hydrophobic barrier[29].

(4) Under normal enviromental conditions and at physiologic pH, the mucin layer overlying the corneal epithelium bears a net negative charge[10,28]. Accordingly, the enhanced binding between positively charged liposomes and the corneal epithelium could be due to electrostatic adsorption, which was based on charge only. Also, Schaeffer and Krohn investigated that liposomes-corneal binding affinity was dependent on the electrostatic adsorption[28].

It appears that the potential advantages of liposomes, as a novel ophthalmic delivery system, include their ability to control the rate of release of the encapsulated drug and their ability to form intimate contact with the corneal epithelium in the ocular tissues.

In summary, these observations revealed that the liposomal ophthalmic drug delivery system seems to be a promising approach to improve the ocular bioavailability and to alter the behaviour or the pharmacokinetics of the encapsulated drug by modifying the drug activity and targeting the drug to the selected site of action.

Table (I): Effect of liposomal surface charge on the encapsulation of brimonidine. Multilamellar liposomes were prepared to contain 12 μmol lipid / ml aqueous phase.

Liposomal surface charge	% Encapsulation*
1 - Positively charged	41.9 (0.6)
2 - Negatively charged	60.5 (0.7)
3 - Neutral	34.8 (0.9)

*The values between parentheses represent the standard error of the mean (n=4)

Table II: Values for the duration of action, area above intraocular pressure/ time curve, maximum response,and time of maximum response brimonidine in solution and in different liposomal formulations.

Formulations	Parameters of activity			
	Duration of action (hr.)	Area above the curve (mm Hg hr.)	Maximum response (mm Hg hr.)	Time of maximum response (hr.)
A- Solution:.	14.00	22.26	6.50	2.00
B-Multilamellar liposomes:				
1- Positively charged	31.00	35.98	11.79	10.00
2-Negatively charged	26.00	27.25	8.60	5
3- Neutral.	29.00	29.55	9.72	5

*The values between parentheses represent the standard error of the mean (n = 6)

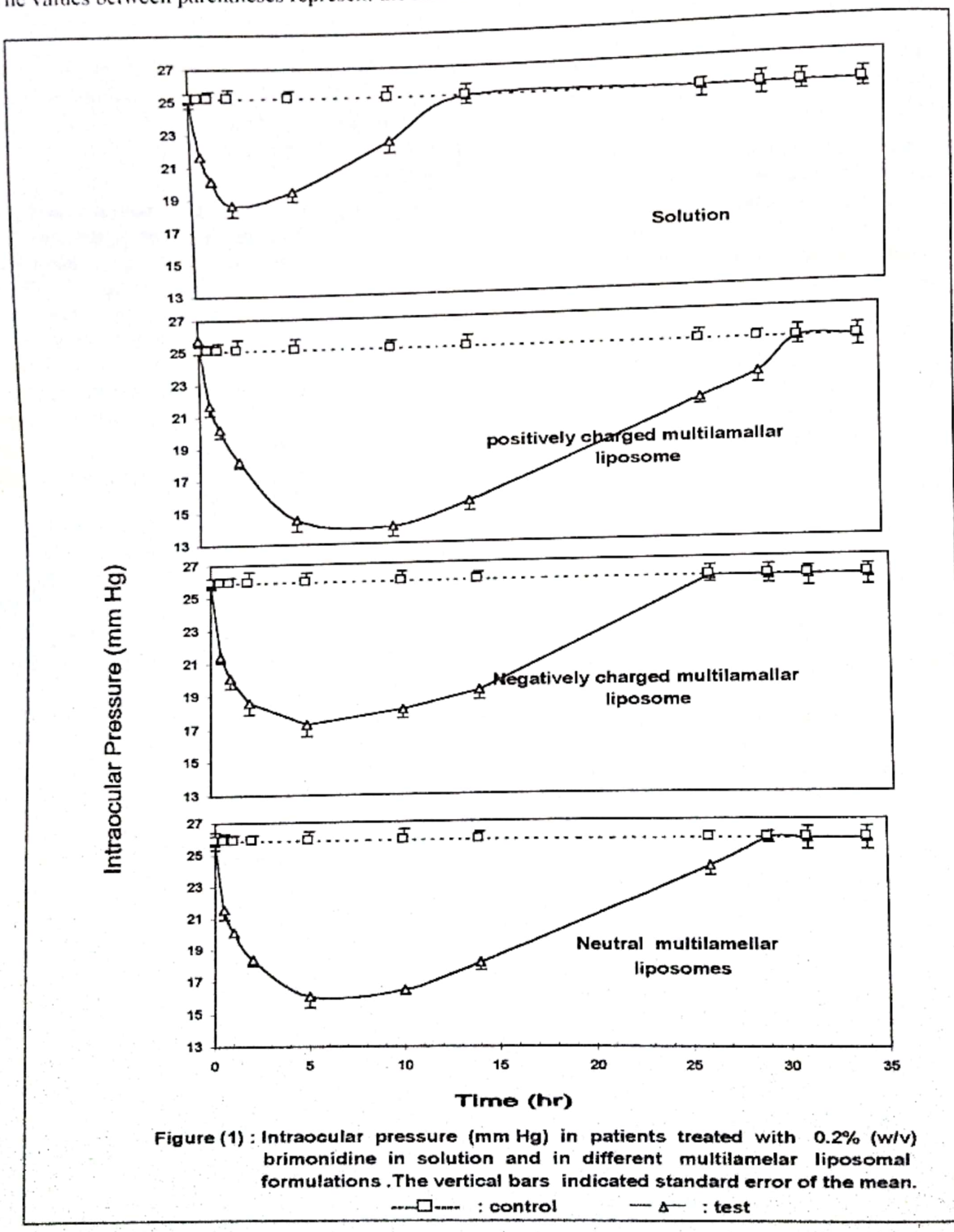

Figure (1) : Intraocular pressure (mm Hg) in patients treated with 0.2% (w/v) brimonidine in solution and in different multilamelar liposomal formulations .The vertical bars indicated standard error of the mean.

---□---- : control —Δ— : test

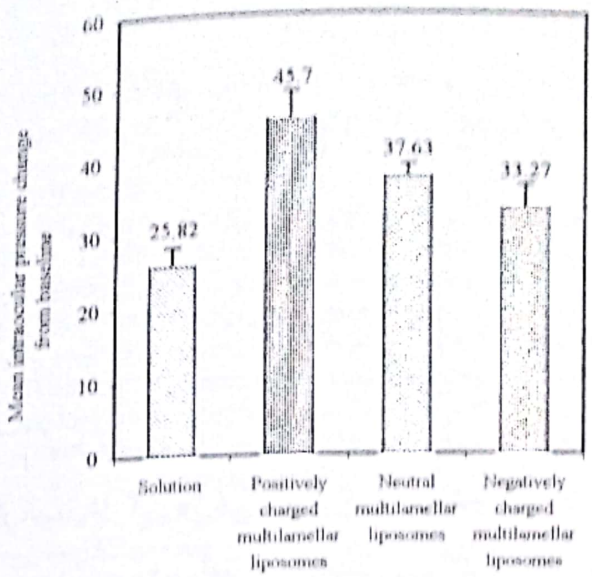

Fig. 2: Mean change in intraocular pressure from baseline in patients treated with brimonidine compared with liposmal treated patients. A complete description on the methods and statistical analysis has been reported in this study.

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استخدام الليبوزوم كحامل مميز للعقار فى تأثير البريمونيدين فى العين

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تم تقييم فاعلية البريمونيدين كمستحضر فى صيغة محلول وكذلك على هيئة ليبوزوم فى جرعة تركيزها ٠,٢ % فى عيون ٢٨ مريضا بالغا ممن يعانون من جلوكوما أولية ذات الزاوية المفتوحة. ولقد حضر الليبوزوم المتعدد الطبقات من الداي فوسفاتيديل كولين والكولستيرول مع إضافة الأستياريل أمين أو داى سيتيل فوسفات لتعديل الشحنة على سطح الليبوزوم. وتم تقييم فاعلية المستحضرات المختلفة للعقار على اساس قياس تأثير العقار على الضغط الداخلى للعين.

أظهرت النتائج أن العقار فى حالة المستحضرات الليبوزومية المختلفة كان له فاعلية أكبر من فاعلية العقار عند وجوده على هيئة محلول. وفضلا عن ذلك واعتمادا على نوع الشحنة الموجودة على سطح الليبوزوم فان الليبوزوم ذو الشحنة الموجبة كان له اكبر فاعلية لتأثير العقار من كل من المستحضرات الليبوزومية الأخرى وكانت أقلها فى حالة الليبوزوم ذو الشحنة السالبة. وعلل ذلك على أساس التجاذب الكهروستاتيكى للشحنة السالبة الموجودة على سطح قرنية العين والشحنة الموجبة الموجودة على سطح الليبوزوم. ويستنتج من ذلك بوضوح أن الليبوزوم كنظام ناقل للعقار يعتبر وسيلة ناجحة للتصويب العقار فى العين.

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