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A Long Acting Ophthalmic Gel Formulations of Atenolol

Maha A. Hassan
Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Asyut, Egypt

The main aim of pharmacotherapeutics, is the attainment of an effective drug concentration at the intended site of action for a sufficient period of time to elicit the response. In this study a trial was made to formulate atenolol, which is a beta-adrenergic blocker in a topical ophthalmic gel. Two polymers were used in this study, carboxymethylcellulose and sodium alginate in different concentrations. Atenolol was used in concentrations 0.5, 1, and 1.5% w/v. The in vitro release study was carried out. The results showed that the release rate of atenolol from gel preparations decreased as an inverse function of polymer concentration, while the release rate of the drug increased as the initial concentration increased. The data of drug release from the two polymers in different concentrations was plotted against the square root of time, and the diffusion coefficients (D), were calculated from the slope of the equation.

Intra-ocular pressure (IOP) measurements of the rabbit's eye treated with 1% w/v atenolol solution, and 1% w/v atenolol in two gel formulations with different concentrations of the polymer were determined. The two gel formulations showed that these polymers extended the duration of pressure reducing effect to 8 hr, when compared with atenolol solution. Area above the curve (AAC), maximum response, maximum time of response (t_{max}), and the duration of action were calculated.

The overall results of this study indicated that the gel formulations of atenolol could be used for the development of a long-acting ophthalmic formulation.

Keywords atenolol; gel; topical preparations

INTRODUCTION

The major problem being faced in ocular therapeutics is the attainment of an optimal concentration at the site of action. Poor bioavailability of drugs from ocular dosage forms is mainly due to tear production, non productive absorption, transient residence time, and impermeability of corneal epithelium (Kaur et al., 2002; Sechoy et al., 2000).

Due to these physiological and anatomical constraints only a small fraction of the drug, effectively 1% or even less of the instilled dose, is ocularly absorbed. The effective dose of medication administered ophthalmically may be altered by varying the strength, volume, or frequency of administration of the medication in contact with the surface of the eye. So far, attempts have been made to improve ocular drug bioavailability by extending drug residence time in the conjunctival sac and improving drug penetration across the cornea, the major pathway of drug entry into the internal eye (Kaur et al., 2002).

As the ocular efficacy of topically applied drugs is influenced by the corneal contact time, the most common method of improving the ocular availability of drugs is to increase the precorneal residence time by using hydrogels (Edsman et al., 1996; Fitzgerated & Wilson, 1994).

Several ways of prolonging the presence of drugs in precorneal area consist of increasing the viscoisty of the dosage form by adding a number of water soluble or insoluble, natural, synthetic, and semisynthetic polymers (El-Kamel, 2002; Felt et al., 1999; Lin & Sung, 2000; Thermes et al., 1992; Xu et al., 2002; Yie et al., 2000). However, these polymers explicit characteristics such as high viscosity, ensuring prolonged retention and a better miscibility with the lacrymal fluid, which helps in release of water soluble drugs. Moreover, these agents are usually biocompatible.

Alginates are established among the most versatile biopolymers, used in wide range of applications. The conventional use of alginate as an excipient in drug products generally depends on the thickening gel forming and stabilizing properties. Hydrocolloids like alginate can play a significant role in the design of controlled-release products (Tonnesen & Karlsen, 2002).

The aim of this work is the formulation and evaluation of atenolol ophthalmic gel. The formulation variables that could affect the release rate and absorption of the drug in topical formulations, such as the polymer type, concentration of gelling agent, and the initial drug concentration in the formulations, were studied. In addition the in vivo performance of gel formulations of atenolol was assessed on the basis of the influence of the drug on the intra-ocular pressure of rabbit's eye, and the duration of pressure reducing effect.

EXPERIMENTAL

Materials

Atenolol was purchased from Fishcer chemicals AG (Riesbachstr. 57, 8034 Zurich/Switzerland), sodium alginate

Address correspondence to Maha A. Hassan, Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt. E-mail: mahashawkat@yahoo.com

(PRONOVA biopolymer, Norway), carboxymethylcellulose from (Dow Chemical Company, Midland, Mich, USA). All other chemicals were of analytical grade.

Apparatus

- pH meter (Precision ama-digital 140 pH, Germany)
- Stirrer (Gallenhamp Magnetic Stirrer Hotplate 400, United Kingdom).
- Thermostatically controlled mechanical shaker water bath (Unitronic, 32-OR, Spain).
- Double beam spectrophotometer (Shimadzu, UV-150-02, Japan).
- Schiotz tonometer (Riester, Germany).
- Brookfield DV±II model LV Viscometer (USA).

Methods

Preparation of Atenolol Solutions

Sterile aqueous atenolol solutions with the concentrations 0.5, 1, and 1.5% w/v were prepared using sterile water as a vehicle. Sodium hydroxide solution (0.1 N) was added to adjust the pH at 6.8. Benzalkonium chloride (0.005%) was also added as a preservative, and the solutions were made appropriately isotonic with sodium chloride (Roebling osmometer), and then filtered through 0.45 μm filter prior to use. The same solution was prepared without the drug to be used as a control.

Preparation of Atenolol Gel

Different concentrations of atenolol (0.5, 1, and 1.5% w/v) were dissolved in sterile water. A weighed amount of sodium alginate or carboxymethylcellulose was gently added to the drug solutions in a beaker and mixed thoroughly using magnetic stirrer and a bar. 0.1 N sodium hydroxide solutions were added to adjust the pH of the prepared polymer solution, benzalkonium chloride (0.005%) as a preservative and sodium chloride to adjust the tonicity were added. The container was left overnight to ensure complete mixing. Eventually, a clear viscous gel formed. Gel formulations were prepared one day prior to use and a separate formulation without the drug (as control) was prepared for each experiment.

Content Uniformity

One gram of the gel prepared from each concentration of the drug, and from each polymer concentration was dissolved in 100 ml phosphate buffer (pH 6.8), then an aliquot of 5 ml was withdrawn and filtered through nylon membrane filter (0.45 μm). The filterate was diluted with the same buffer, and the concentration of the drug was determined spectrophotometrically at 275 nm. Each determination of each concentration was repeated three times. The percentage of drug content was between 98–101%. Only samples with drug content of 98% were used for diffusion studies.

Determination of Viscosity of Atenolol

The viscosity of atenolol gel bases was determined using (Brookfield DV±II model LV viscometer) at temperature 37 ± 1°C using Helipath stand and T-bar spindle. Fifty grams of the sample was tested using a 100 ml capacity vessel. The viscosity was determined at intermediate speed (1.5 rpm). The readings were taken after a specified time (5 min).

In Vitro Release Studies

The in vitro release of atenolol from solutions or gel formulations was carried out using dialysis method. A semipermeable standard cellophane membrane was stretched over the open end of 2 cm diameter glass tube. The membrane was made watertight by a rubber band. 1 g of atenolol solution, or different gel formulations was placed in the tube, which was suspended so that the membrane was just below the surface of buffer solution. The volume of phosphate buffer (pH 6.8) used as release medium is 100 ml at 37 ± 1°C. Samples each of 5 ml were withdrawn from the beaker content at different time interval, and measured spectrophotometrically at 275 nm. The removed samples (5 ml) were replaced by equal volumes (5 ml) of phosphate buffer. Each in vitro diffusion test was repeated three times.

In Vivo Studies

Four adults male albino rabbits, weight range 1.5–2 kg were participated in the study. The animals were kept in individual cages with free access to food and water. One week at least elapsed between testing in the same rabbit. Xylocaine solution (2%) was dropped into the rabbit's eyes to anaesthetize the cornea. Ophthalmic drug solution or gel formulations were applied into the right eye, where the left eye used as control. During applications, the upper eyelid was slightly raised and the lower eyelid was gently pulled away. A 100 mg dose of solution or each gel formulation was applied directly into the conjunctival sac of the eye. At certain time intervals, the intra-ocular pressure (IOP) was measured before and after application of the formulations for both control and tested eyes using Schiotz tonometer. At least three individual successive measurements were made at each time. The mean of the three consecutive tonometeric measurements was calculated for each sample. The IOP was measured at different time interval; until the IOP returned to the basal line. The area above the curve (AAC) was measured for 8 hr, maximum response, time of maximum response (t_{max}) and duration of action was determined.

Treatment of Data

A Higuchi equation was used to analyze the release data of atenolol from the different gel formulations:

$$Q = 2Co\sqrt{\frac{Dt}{\pi}}$$

where ***Q*** is the amount of drug released per unit area (mg/cm^2), ***Co*** is the initial drug concentration in the vehicle (mg/cm^2), ***D*** is the diffusion coefficient (cm^2/hr), and ***t*** is the time. The data of the drug release from the formulations was plotted against the square root of time ($\sqrt{t}$), and the diffusion coefficient (D), were calculated from the slope of equation.

Statistical Analysis

The results were presented as mean ± *SD*. The data of atenolol effect on IOP of rabbit's eyes from different concentrations of CMC and sodium alginate gel were compared with atenolol solution using *t*-test, and ANOVA test.

RESULTS AND DISCUSSION

In Vitro Release Studies

Effect of Polymer Concentration on Drug Release

The release of atenolol from the gels of three varying polymer concentrations (1, 2, 3% w/v of CMC, and 5, 10, 15% w/v of sodium alginate) was evaluated. The results showed that the release rate of atenolol from the gel preparations decreases as an inverse function of polymer concentration. As shown in Figures. 1–6 when the cumulative amounts of atenolol released were plotted as a function of the square root of time, linear correlations (Table 1) were observed, indicating that the release of drug from the vehicle was in compliance with the Higuchi diffusion model described for the release of drugs from the semisolid base. The diffusion coefficient values calculated from Higuchi plots for the different vehicles are shown in Table 1, and were found to decrease inversely as a function of polymer concentration (Demou et al., 1994). In our study the finding that higher polymer concentrations resulted in lower drug release from the vehicles is in agreement with Kassem et al. (2002), which stated that the diffusion coefficient of a solute is inversely proportional to the volume fraction occupied by the gel-forming agent (El-Gendy et al., 2002).

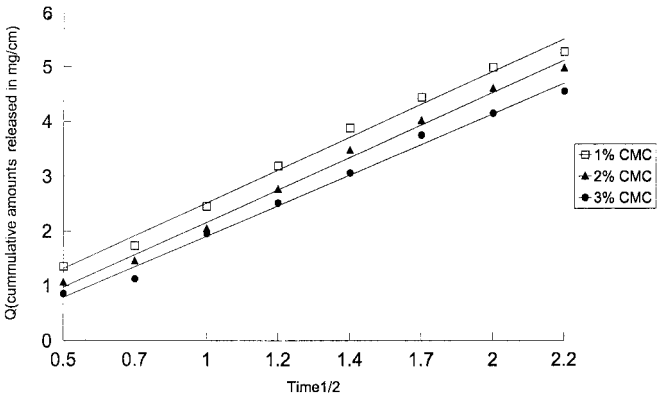

FIGURE 1. The cummulative amounts released of 0.5% w/v atenolol in different concentrations of CMC gel.

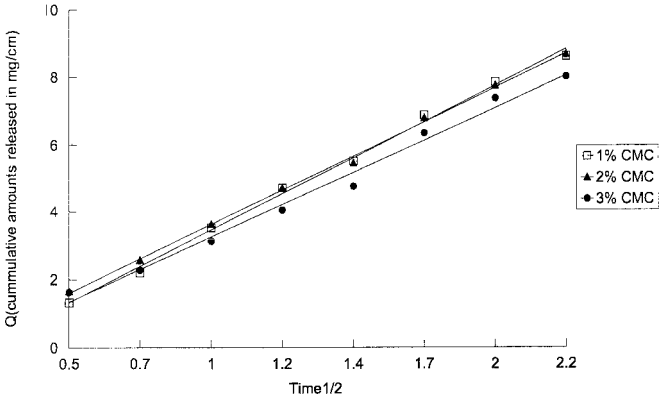

FIGURE 2. The cummulative amounts released of 1% w/v atenolol in different concentrations of CMC gel.

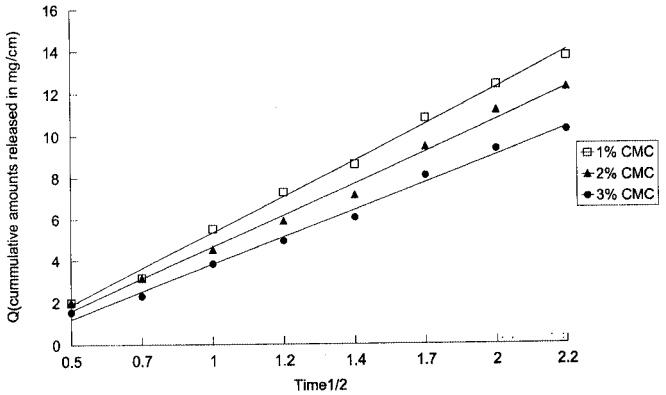

FIGURE 3. The cummulative amounts released of 1.5% w/v atenolol in different concentrations of CMC gel.

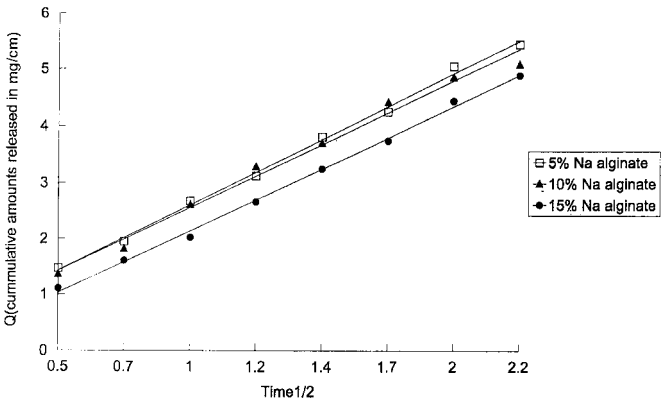

FIGURE 4. The cummulative amounts released of 0.5% w/v atenolol in different concentrations of sodium alginate gel.

Figures from 1–6 showed the cumulative amounts (Q) of atenolol released from both gel polymers with different concentrations against the square root of time √t. Figures (1–3) showed that the cumulative amounts released of atenolol from CMC gel. From the figures it was observed that at 0.5% w/v

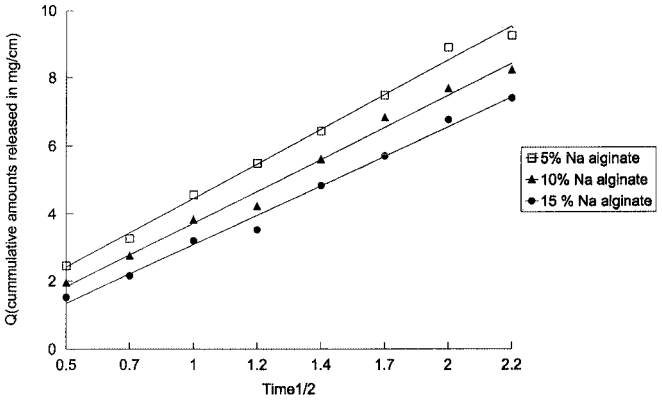

FIGURE 5. The cummulative amounts released of 1% w/v atenolol in different concentrations of sodium alginate gel.

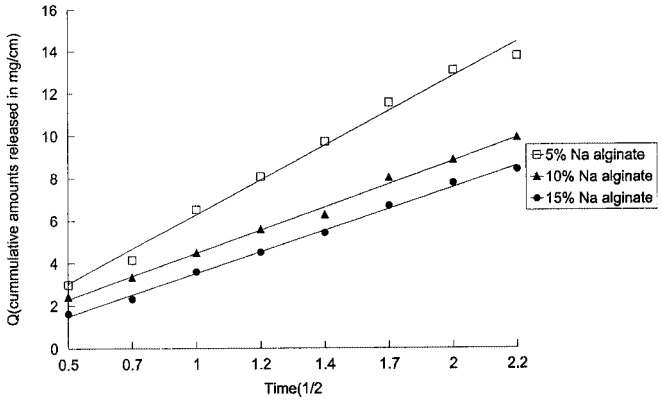

FIGURE 6. The cummulative amounts released of 1.5% w/v atenolol in different concentrations of sodium alginate gel.

atenolol concentration the amounts released are 5.3, 5, and 4.6 mg from 1, 2, and 3% w/v CMC, 8.6, 8.1 and 8 mgs from 1% w/v atenolol and 13.7, 12.2, and 10.2 mg from 1.5% w/v atenolol with the same concentrations of CMC gel (1, 2, and 3% w/v). Figures 4–6, also represent the cumulative amounts released of different concentrations of atenolol from sodium alginate gel. The results showed that at 0.5% w/v atenolol, the cumulative amounts released were 5.5, 5.1, and 4.9 mg, at 1% w/v atenolol concentration, the cumulative amounts were 9.3, 8.2, and 7.4 mg, and at 1.5% w/v atenolol concentration were 13.7, 9.9, and 8.4 mg from the different concentrations of sodium alginate gel (5, 10, and 15% w/v). Table 1 shows the diffusion coefficient (D). From the table it was observed that as the concentration of the polymer increases, the diffusion coefficient decreases for the same concentration of the drug. For example the diffusion coefficient of 1.5% w/v atenolol is 0.689, 0.462, and 0.430 cm^2/hr for 5, 10, and 15% w/v sodium alginate gel. The same results obtained with CMC gel, it was observed that the diffusion coefficient is 0.733, 0.646, and 0.555 cm^2/hr for 1.5% atenolol in 1, 2, and 3% w/v CMC gel.

The diffusivity of the drug through any base depends on the nature and composition of the individual base (Babar et al., 1990). A change of polymer concentration in these two gels could affect the diffusional pathways and thus drug release. Therefore, the fact that hydrogels have shown a good release of the drug might be related to the ease of drug diffusion through the aqueous channels or diffusional pathways formed within the gel network (Babar et al., 1990).

Effect of Drug Loading on Release

The effect of the initial drug concentration on the drug release was evaluated. Figures 1–6 showed the release of different drug concentration from the two different gel bases. The data showed that the release of atenolol from the two gel bases increases with an increase of the initial load in the vehicles. The figures showed the cumulative amount (Q) released form different concentration of atenolol against the time. It was observed that the amount released of 0.5, 1, and 1.5% w/v from 10% w/v sodium alginate is 5.1, 8.2, and 9.9 mg, respectively, and the amounts released of the drug from 2% w/v CMC are 5, 8.1, and 12.2 mg from 0.5, 1, and 1.5% w/v of atenolol, respectively. These results indicated that as the concentration of the initial drug increases, the release of the drug increases. This effect is probably due to the increase in thermodynamic activity of the drug, which is related to its concentration in the base (El-Gendy et al., 2002). In the present work, the release was increased at all levels of drug concentration used. Also, the increase in release profiles with increment in the loading dose may be due to the higher availability of the drug on the release surface. Almost all formulations have shown a linear relationship (Table 1) when the amount of drug released is plotted against the square root of time, as shown in Figures. 1–6.

The In Vivo Performance of Atenolol

The drug activity was investigated by IOP lowering effect produced by the beta-adrenergic blocking activity of the drug. The area above the IOP/time curve, the maximum response, time of maximum response, and the duration of drug action were the parameters, which had been taken in consideration to quantify the drug activity. The values of these parameters were calculated and summarized in Table 2. It was observed that these parameters were different among the different preparations, and influenced by the type and concentration of polymer used in gel formulation. The time against the IOP of solution and the different gel formulation are represented in Figures. 7, 8. From the figures, it was observed that the time for the determination of IOP was 8 hr, and the duration of action on IOP increase as the concentration of polymer increase in both formulations sodium alginate and CMC gel of atenolol when compared with atenolol solution. Also, the figures showed that the decrease of IOP increase in order 3% CMC > 2% CMC > 1% CMC solution respectively, and in sodium alginate gel

TABLE 1
Effect of Vehicle Concentration on the Diffusion Coefficient (D) of Atenolol Through Cellophane Membrane

Vehicle Concentrations (% w/v)	Atenolol Concentrations (% w/v)	Higuchi Diffusion Correlation (r)	Diffusion Coefficient (D) (cm^2/hr)
Sodium alginate	0.5%		
5%		0.992	0.738
10%		0.998	0.715
15%		0.998	0.703
Sodium alginate	1%		
5%		0.998	0.648
10%		0.995	0.599
15%		0.999	0.557
Sodium alginate	1.5%		
5%		0.994	0.689
10%		0.998	0.462
15%		0.999	0.430
CMC	0.5%		
1%		0.993	0.762
2%		0.995	0.753
3%		0.995	0.711
CMC	1%		
1%		0.991	0.633
2%		0.994	0.600
3%		0.992	0.567
CMC	1.5%		
1%		0.998	0.733
2%		0.998	0.646
3%		0.998	0.555

TABLE 2
Values of Area Above the IOP/time Curve, Duration of Action, Maximum Response, Time of Maximum Response of 1% w/v Atenolol of Both Solution and Gel Formulations

Gel Formulations (% w/v)	AAC (mmHg.hr)	Maximum Response (mmHg)	t_{max} (hr)	Duration of Action (hr)
Atenolol solution	8.4	6.1	1	3
Sodium alginate				
5%	13.9	7.2	2	4
10%	19.8	7.2	2	5
15%	27.0	8.2	3	6
Carboxymethyl cellulose (CMC)				
1%	24.6	7.2	2	6
2%	36.2	8.2	4	7
3%	45.4	9.2	4	8

15% sodium alginate > 10% sodium alginate > 5% sodium alginate > solution respectively.

Duration of Drug Action

The duration of intra-ocular pressure reducing effect of 1% w/v atenolol formulated in solution or other gel formulations could be seen in Table 2. The most prolonged effect could be achieved using the high concentration of the two polymers used (CMC and sodium alginate), while the shortest duration obtained by using the low concentrations of both polymers.

From statistical analysis, ANOVA test showed significant difference at $P > 0.01$ in the decrease of IOP for different concentrations of CMC and sodium alginate gel of atenolol in comparison with the atenolol solution and significant difference at $P > 0.05$ in the decrease of IOP between the different concentrations of CMC and sodium alginate gel of atenolol. *t*-test showed significant difference at $P > 0.05$ for the decrease of IOP for different concentrations of CMC and sodium alginate gel of atenolol in comparison with the atenolol solution and significant difference at $P > 0.05$ between the different

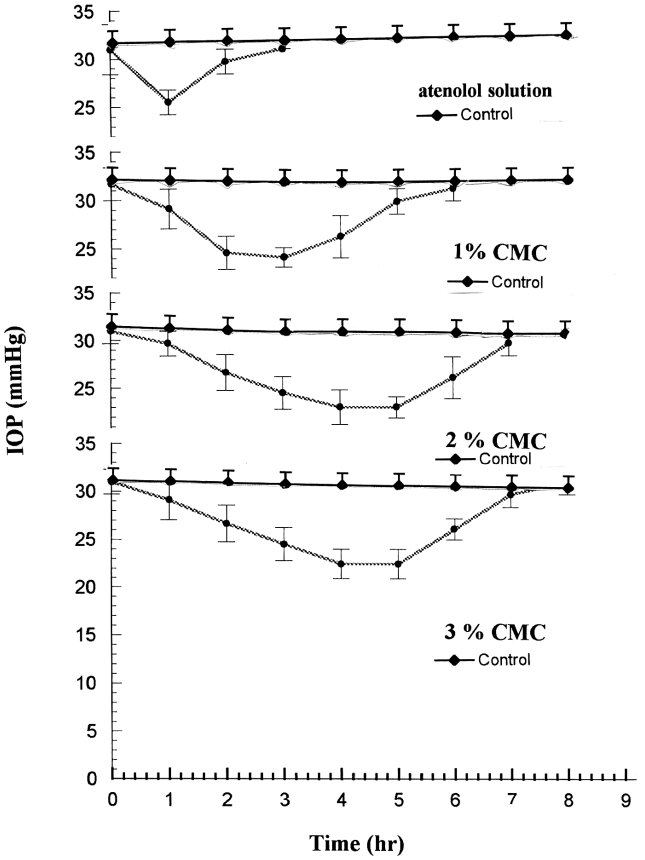

FIGURE 7. Intra-ocular pressure (mmHg) of rabbit's eye after instillation of 1% w/v atenolol in solution and in different concentrations of CMC gel.

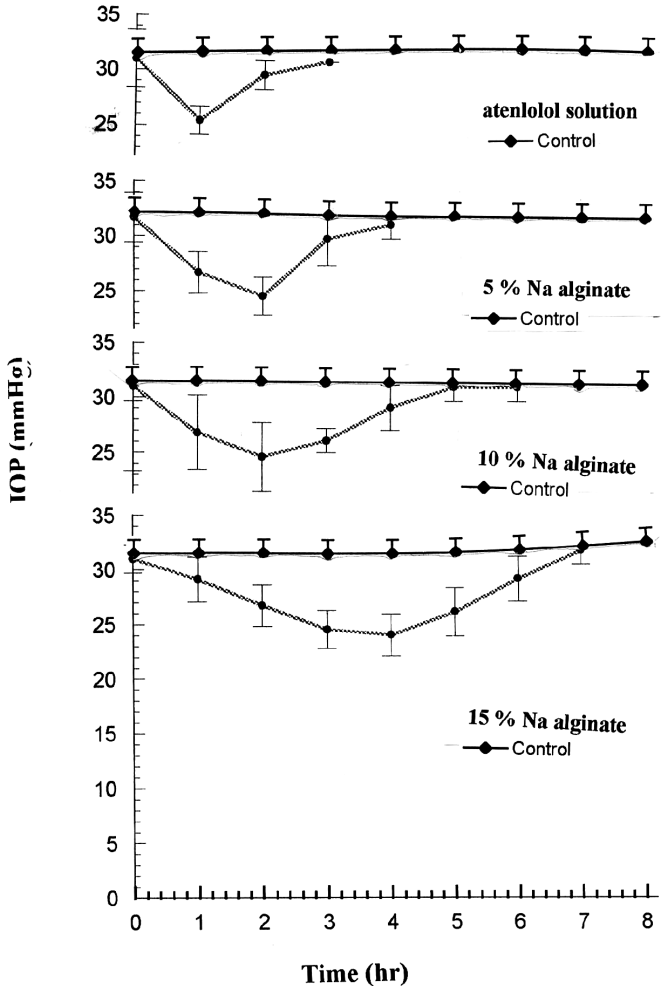

FIGURE 8. Intra-ocular pressure (mmHg) of rabbit's eye after instillation of 1% w/v atenolol in solution and in different concentrations of sodium alginate gel.

concentrations of both gel formulations. Duration of action as long as 8 hr was recorded for CMC gel formulation at concentration 3% w/v, while the duration of action is 6 hr for 15% w/v sodium alginate. The shortest duration of action was observed with 5% w/v sodium alginate gel (Table 2). These results attributed to the concentration of the polymer used as the concentration of the polymer increases the viscosity increases. The results of the viscosity indicated that the viscosity of 5, 10, and 15% w/v Na alginate are 6800, 16200, and 23000 cps respectively, while the viscosity of 1, 2, and 3% w/v CMC are 36000, 53200, and 68000 cps, respectively. The higher concentration of the polymer lead to long contact time of the drug formulation to the cornea of eye, and extending the drug residence time in the conjunctival sac and improving the drug penetration across the cornea.

Sodium alginate is known as a bioadhesive polymer in topical delivery. In a neutral medium the mucin molecule is negatively charged (pKa=2.6) and behaves as an anionic polyelectrolyte forming a weak viscoelastic gel, which consists of network of linear, flexible, and random coil molecules (Albasini & Ludwing, 1995). The mucoadhesive properties of the polymers might therefore, influence contact times of vehicles. How well the gel stays in the eye is probably dependent not only on its mucoadhesive properties, but also on its bulk rheological properties (Sechoy et al., 2000).

Maximum Response and Time of Maximum Response

Table 2 represents the measured values for the maximum response and its time. From the table it was observed that an increase in the maximum response of atenolol could by achieved by increasing the viscosity of gel in both formulations. The highest increase in the maximum response could be achieved by the highest viscosity of the polymers 15% and 3% w/v for sodium alginate and CMC, respectively.

The time of maximum response showed an agreement with maximum response. The measured time of maximum response represents that the longest time obtained with the highest concentration of the two polymers, while the shortest time of maximum response appear with the lowest concentration of both formulations. The increase in the maximum response obtained

by the higher concentration of polymers (high viscosity) could be explained by increased contact time for the drug to the corneal tissues, which give better chances for higher drug absorption.

The area above the curve (AAC) was determined for different preparations (solution and gel formulations) for 8 hr. From Table 2, it was observed that the AAC of 1% w/v atenolol increase as the concentration of the polymer increase. In sodium alginate gel at concentration 15% w/v, AAC is 27 mmHg.hr, while at concentration 5% w/v AAC is 13.9 mmHg.hr. The same results obtained with CMC gel, it was observed that the AAC is 24.6 mmHg.hr for 1% w/v atenolol in 1% w/v CMC gel, while the AAC of 3% w/v CMC gel is 45.4 mmHg.hr. From the table also it was observed that atenolol solution give the lowest AAC, which is 8.4 mmHg.hr.

From the above-mentioned results, it should be noted that the gel formulations of the drug with high viscosity have a greater influence on the parameters of the drug action compared to that with low viscosity. Then the higher viscosity produced greater activity of the drug compared to the lower concentration of the polymer (low viscosity). These results can explained on the basis that the higher concentration of the polymer controlled the drug release and lead to prolongation of ocular contact time and help in drug penetration into ocular tissues, which increase the drug duration of action and consequently the bioavailability.

CONCLUSION

From the above mentioned results, it should noted that, the in vitro study is considered as useful methodology which could provide a base for developing topical dosage form of the drug having desirable release profile. The atenolol gel formulations were made and examined under different formulations variables. The nature of the polymer, and its concentration has influenced drug release profiles. Higher release rates were observed at lower polymer concentrations. An inverse relationship was observed between the polymer concentration and the diffusion coefficient. Intra-ocular pressure (IOP) measurements of rabbit's eyes treated with 1% w/v atenolol gel formulations, showed that these polymers extended the duration of the pressure-reducing effect of atenolol to 8 h. These results attributed to the prolongation of ocular contact time and help the drug penetration into ocular tissue, which increase the drug duration of action and consequently the bioavailability.

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