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BIOAVAILABILITY OF CARBACHOL CHLORIDE FROM DIFFERENT GEL FORMULATIONS

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ABSTRACT

The in vitro release of carbachol chloride, a cholinomimetic drug, from different ophthalmic gel-formulations has been investigated. The release data were found to be in favour of the diffusion controlled mechanism. The effect of viscosity on the release pattern has been also studied. The amount of the drug diffused from the aqueous solution of the drug was found to decrease with an increase in the solution viscosity. On the other hand, the viscosity did not affect the release rate when the drug was formulated in gels.

The effect of different gel formulations on the drug activity, a decrease in the intraocular pressure, IOP, was studied on human patients. It was found that the incorporation of the drug into hydrophilic gels resulted in an improvement of its activity. The parameters of drug activity are:- area above time curve; maximum drug response and time of maximum response. The statistical analysis of the data obtained was assessed. A correlation between the in vitro and in vivo data has been deduced.

INTRODUCTION

Carbachol cholride is a cholinomimetic drug. However it is more stable and shows a considerable selectivity of action. At present it is used only for its miotic action in the treatment of primary glaucoma. Untoward effects, from ophthalmologic use, include, celiary spasm and headache. The celiary spasm may result in a temporary decrease in visual acuity. Sufficient systemic absorption sometimes occurs to cause systemic effects.

Many techniques have been utilized to enhance the bioavailability of drugs which are delivered topically to the eye. Eye drops still represent the major form for the topical ocular route. The presence of tears in

the culde-sac and the turnover of lacrimal fluid. $16\%/min^{-1}$, in man [1] can cause a significant loss of the topically applied drug when instilled as an ophthalmic solution. In addition, the efficient drainage apparatus, used for removal of tears, aggravates the loss of drug from the precorneal area [2]. The previously mentioned factors are considered as the main drawbacks for the use of drops as ophthalmic medications. Also, the oleaginous and the oily formulations undergo from some therapeutic disadvantages [3&4].

Results of previous studies, on rabbits and humans, indicate that aqueous-based gels appeared to offer advantages over the traditional ophthalmic dosage forms, either in terms of improved ocular drug bioavailability [5] or enhanced therapeutic response [6]. Many of the ophthalmic gels, investigated up till now, have been formulated with either carbomers, cellulose derivatives [7] and ploxamer 407 [8]. Ploxamers, a class of gel-forming polymers, have been evaluated as a semi solid vehicles and possess several properties which appear to make them particularly suitable for use in the formulation of ophthalmic dosage forms, including their low toxicity, mucomimetic properties and optical clarity [9].

It is well known that carbachol is commonly used in concentrations of 0.75% to 3% for the treatment of the open angle glaucoma. It is recommended for those patients where the glaucoma can not be controlled with pilocarpine therapy [10]. The major disadvantage of carbachol is its short duration of action, 2-3 hours [11].

The purpose of the present investigation was to formulate carbachol chloride in different gel-forming polymers with the aim to prolong its short duration of action. Also, the study was carried out to elucidate: i) the effect of some gel-forming vehicles on the release profile of the drug; ii) the *in vivo* performance of carbachol chloride ophthalmic gels in human eyes, the decrease in IOP, and iii) the correlation between the *in vitro* and *in vivo* performance of the drug.

EXPERIMENTAL

Materials

Carbachol chloride (M.S.D., U.S.A.), Methylcellulose 450 (BDH, U.K.), Methylhydroxycellulose, Tylose 4000 (Hoechst, W. Germany), Carbomer 934, Carbomer 940 and Carbomer 941 (B.F. Goodrich Co., Cleveland, Ohio, U.S.A.), Ploxamer 407 (BASF, Wyandotte, MI, U.S.A., Benoxinate HCl (Dr. Thilo & Co. GMBH, Germany) and semipermeable standard cellophane membrane (30/32, Fischer Sci., London, U.K.). All the other chemicals used were of analytical grade.

Equipment:

Shaking water bath (Selects, Uniform 320), Double beam spectrophotometer (Shimadzu, Japan), Rotational viscometer (RN-MLW, Medingen, GDR) and Schiotz tonometer (John Weiss & Son Ltd, WIH ODN, London, U.K.).

Procedure:

Preparation of Ophthalmic Gels:

All the tested ophthalmic gels were prepared in such a manner to contain carbachol chloride in 1.5% w/w concentration. The same concentration was used to prepare the aqueous solution.

1- Ploxamer Gel:

An accurate weight of carbachol chloride, 1.5 g, was placed into a 100 ml conical flask and was dissolved in a suitable volume of Sorensen's phosphate buffer at pH 7.4. The required amount of the ploxamer was dispersed into the drug solution. The flask was stored at 5°C for 24-36 hours until the polymer has been completely dissolved. The resultant solution was completed to 100 g with the same buffer solution and thoroughly mixed while cold. When the mixture was warmed to room temperature, a clear viscous gel was produced.

2- Carbomer Gels:

Two grams of carbomer powder were dissolved in Sorensen's phosphate buffer with the aid of a high speed mechanical stirrer. The solution was neutralized with sodium hydroxide solution (400 mg NaOH/1g carbomer). An accurate weight of the drug 1.5 g, was dissolved in about 5 ml of the buffer and incorporated into the previously prepared gel. The total weight of the prepared gel was completed to 100 g with the buffer.

3- Methylcellulose (MC) and Tylose (TY) Gels:

Three and five grams of MC and TY were dissolved in hot isotonic buffer, respectively. Solutions of the drug (1.5 g in about 5 ml buffer) were incorporated into the prepared gel and the weight of the gel was completed to 100 g with the same buffer solution.

Tests Performed on Ophthalmic Gels:

1- *In Vitro* Procedure:

The release of carbachol chloride from the tested gel formulations was followed employing the simple dialysis technique. In this method, two grams of the tested gel formulation were accurately weighed over the semipermeable membrane. The loaded membrane was stretched over one end of an opened glass cylinder, the donor, with an internal diameter of 2.2 cm. The opened upper part of the glass cylinder was covered with a thinly perforated nylon sheet to minimize the possible evaporation of the liquids present in the gels, during the experimental period. The donor was placed inside a 100 ml beaker containing 25 ml of an isotonic buffer at pH 7.4, the acceptor, in such a manner that the membrane was located just below the surface of the sink solution.

The whole dialysis unit was placed into a thermostatically controlled water bath shaker operating at 35°C and 25 shake/minute. One ml was withdrawn from the sink solution at suitable time intervals. The withdrawn volume was replaced by an equal one at the same temperature to keep the volume of the sink constant during the experimental study. The withdrawn samples were assayed for their drug content by measuring the absorbance at 570 nm according to the procedure previously described by Doulakas [12].

2- *In Vivo* Procedure:

Eight patients were enrolled in this study. They were suffering from primary acquired glaucoma, either with the open-angle or narrow-angle type. The enrolled

patients should not under therapy with any antiglaucoma drug. Those patients using antiglaucoma therapy were given a washout period of two weeks before enrolling in the study.

The Intraocular pressure (IOP) was measured in all patients with Schiotz tonometer, Sketch 1. It consists of a curved footplate designed to fit the average normal cornea with a metal plunger in the centre for holding various weights. On the top of the plunger a short curved arm with a lever whose long arm is a pointer for reading positions on a scale. As the plunger idents the cornea, the scale reading will increase according to the resistance encountered. Each instrument is accompanied with a graph that expresses the scale readings in mm Hg of the internal pressure within the eye. After instillation of one drop of the surface anaesthetic benoxinate HCl, 0.4%, the patient is placed in the supine position and asked to look directly upward, fixing on some object such as his extended hand. The lids were separated to keep them away to be in contact with the eyeball, taking care not to exert pressure on the globe with physician's fingers. The instrument is placed gently in a vertical position directly over the cornea and the plunger was allowed to exert its full weight. Three readings were taken using the weights 5.5, 7.5 and 10 grams, and the average IOP was calculated. It is worthy to note that the IOP of the tested eye was measured before topical application of the coded samples, 0.1 g of each, and after 1, 2, 3, and 6 hours of application.

RESULTS AND DISCUSSION

1-*In Vitro* Study:

The in vitro release of carbachol chloride from different gel formulations was carried out at 35°C and the data obtained are surveyed in Table 1. From these data it can be observed that the amount of the drug released was maximum from the aqueous solution of the drug, control, and reduced when the drug was formulated into gels. The release data were checked according to zero order, first order, and the diffusion controlled mechanism. Preference of some mechanisms was based on the correlation coefficient for the parameters involved. Further confirmation for the mechanism was based on the duration of the lag time, the preference will be considered for the mechanism in which no or shorter lag time occurs. Such an approach has already been suggested by Samuelove et al [13]. Further evidence has been provided by plotting log q versus log t; where q is the amount of the drug diffused per unit surface area at time t. In this case a straight line will be obtained with a slope of 0.5 [14].

The mathematical treatment of the release data, Table 1, is in favour of the well known diffusion mechanism. The data revealed that the diffusion coefficient of the drug in case of the control is higher than that in the other tested ophthalmic gels. From the same table, it can be deduced that as the lag time increased, the diffusion coefficient will be decreased. The lag time is an intensive variable which affects the drug absorption from the eye. The presence of a lag time in such experimental studies could be simply at-

tributed to the presence of the semipermeable cellophane membrane which separates the two compartments.

The data also revealed that, the amount of the drug diffused shows a slight dependency on the viscosity of the gel, Table 1. On the other hand, the effect of the viscosity on the amount of the drug diffused may be pronounced at low viscous system, i.e an increase in the viscosity, from 0.018 to 52 poise, results in a significant decrease in the amount of the drug diffused. The same observation has been obtained by Kassem et al [15].

2-*In Vivo* Study:

The data obtained for the *in vivo* study are presented graphically in Fig. 1, where the average values of the IOP were plotted versus the midpoints of the time intervals. From this figure it could be predicted that the effect of the control reaches its initial IOP value after 3 hours, while the other gel formulations do not go back to the initial value and are not terminated throughout the experimental observation period, 6 hours. The measurement of the IOP was carried out before and after application of the drug at specified time intervals and lasts for 6 hours.

The parameters of the drug activity utilized in this study are: i) area above the IOP-time curves (AAC), which is considered as an indication for the amount of drug absorbed; ii) maximum of drug response (MR); which is considered as an indication for the intensity of the drug action; and III) the time of maximum response (TMR). Those parameters are calculated and summarized in Table 2. From this table it could be observed that 3.3-4.8, 1.3-1.8 and 2.3-3.7 fold increase in AAC, MR and TMR was obtained respectively for the gel formulations as compared with the control.

Results of the t test, Table 3, revealed that the differences in areas above the curves between the gel formulations and the control are very highly significant (p=0.001). The only exceptional case is that between MC gel and the control which is highly significant (p=0.01). The differences in MR between the gel formulations and the aqueous solution are statistically insignificant. The only excpetional case is that between carbomer 941 and the control which is highly significant (p=0.01). Also, the differences in TMR are from highly to very highly significant.

These findings are in agreement with that previously obtained by Miller and Donovan [8] who indicated that the miotic activity of pilocarpine was increased 1.9 fold in gel formulations when compared with the control.

The effect of viscosity of the system on the parameters of drug activity is also depicted in Table 2. From this table it could be observed that the effect is highly pronounced between the control and the gel formulations. On the other hand, the viscosity has no or slight effect on the drug activity in case of the gels. The same observations have been reported by Kassem et al [6] when they studied the effect of betamethasone and phenylephrine HCl formulated in gels, on the IOP of rabbit's eye.

In conclusion, it is likely to state that the prolongation of the contact time via incorpo-

Table 1: Mathematical Treatments of the Release Data according to Zero-Order, First-Order and Diffusion Mechanisms for Carbachol Chloride from Different Ophtalmic Gels.

System	Visco-sity (poise)	Zero order	First order		Diffusion mechanisms				
		r	r	lag t (h)	r	lag t (h)	slope log Q vss. log t	% Q	$Dx10^2$ (cm^2/h)
Control	000.018	0.935	0.995	1.26	0.996	0.15	0.468	82.16	17.68
5 % MC gel	193.900	0.968	0.997	2.14	0.998	0.24	0.542	34.24	3.07
2 % Carb. 934 gel	175.400	0.976	0.999	2.36	0.995	0.31	0.641	28.58	2.14
2 % Carb. 941 gel	195.500	0.960	0.992	4.41	0.997	0.42	0.532	21.52	1.21
2 % Carb. 940 gel	190.100	0.985	0.994	3.14	0.990	0.48	0.493	32.14	2.70
20 % Ploxamer 407 gel	052.940	0.964	0.995	3.12	0.996	0.59	0.514	20.32	1.08
3 % TY 4000 gel	185.500	0.979	0.999	4.18	0.995	0.71	0.526	32.46	2.76

r = correlation coefficient; % Q = % drug released after 3 hours and D = diffusion coefficient.

Table 2: Comparison of Area Above Curve (AAC), Maximum Response (MR) and Time of Maximum Response (TMR) to the Viscosity of Ophthalmic Gels of Carbachol Chloride in Human's eye.

System	Visco-sity (poise)	Parameters of IOP activity		
		AAC (mm Hg.h)	MR (mm Hg)	TMR (h)
Control	000.018	25.88(± 3.5)	14.25(±1.90)	1.5(±0.25)
5 % MC gel	193.900	84.99(±10.4)	18.20(±1.80)	4.9(±0.51)
2 % Carb. 934 gel	175.400	93.96(± 9.3)	19.91(±2.12)	5.5(±0.46)
2 % Carb. 941 gel	195.500	99.35(±13.4)	25.01(±3.06)	5.5(±0.46)
20 % Ploxamer 407 gel	052.940	124.23(±21.3)	25.33(±3.90)	3.5(±0.53)

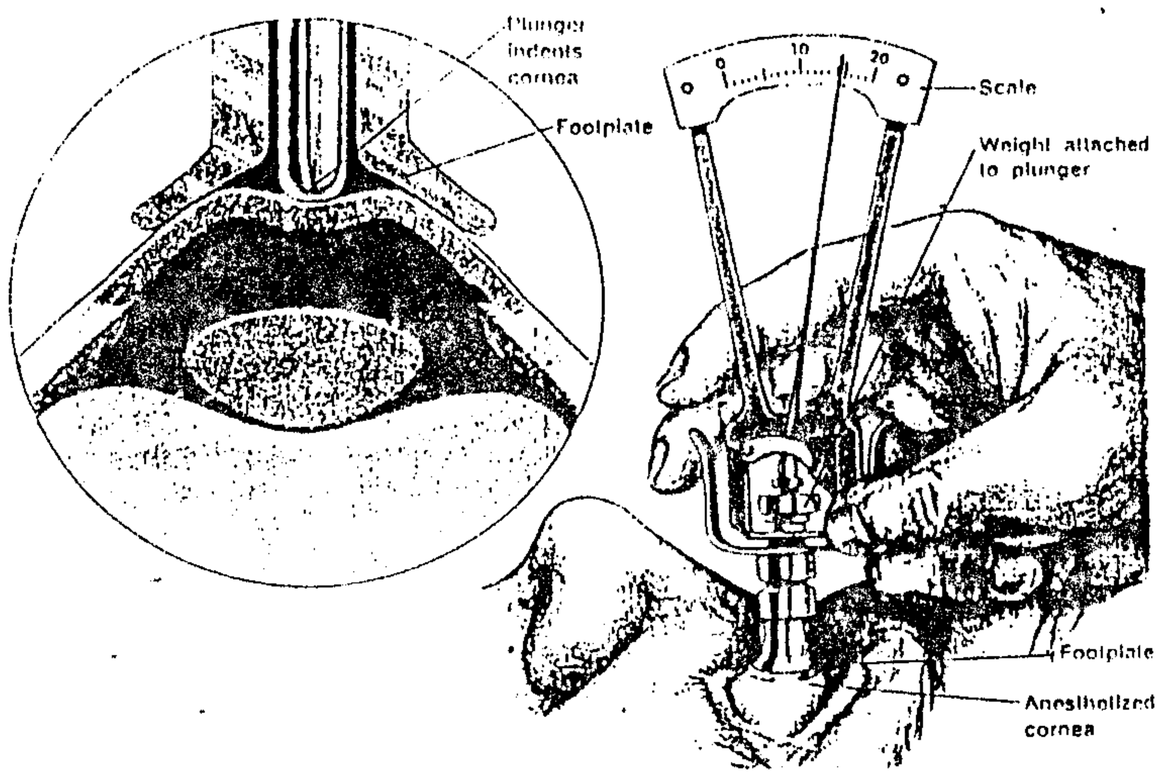
The values between parentheses represent the standered error of the mean.

Table 3: Significance Level (P values) of Differences between Mean Values of the Parameters of IOP Activity of Carbachol Chloride in Human's eye.

Pairs of comparisons	Parameters of IOP activity		
	AAC (mm Hg.h)	MR (mm Hg)	TMR (h)
Control with MC gel	0.010	0.10	0.010
Control with Carb. 934 gel	0.001	0.10	0.001
Control with Carb. 941 gel	0.001	0.01	0.001
Control with Ploxamer 407 gel	0.001	0.10	0.010

The values 0.001, 0.01, 0.05 and 0.1 represent the values of P and they are: very highly significant, highly significent, significant and insignificant respectively.

Bioavailability of Carbachol Chloride from Different Gel Formulations



Sketch 1. Schiotz Tonometer

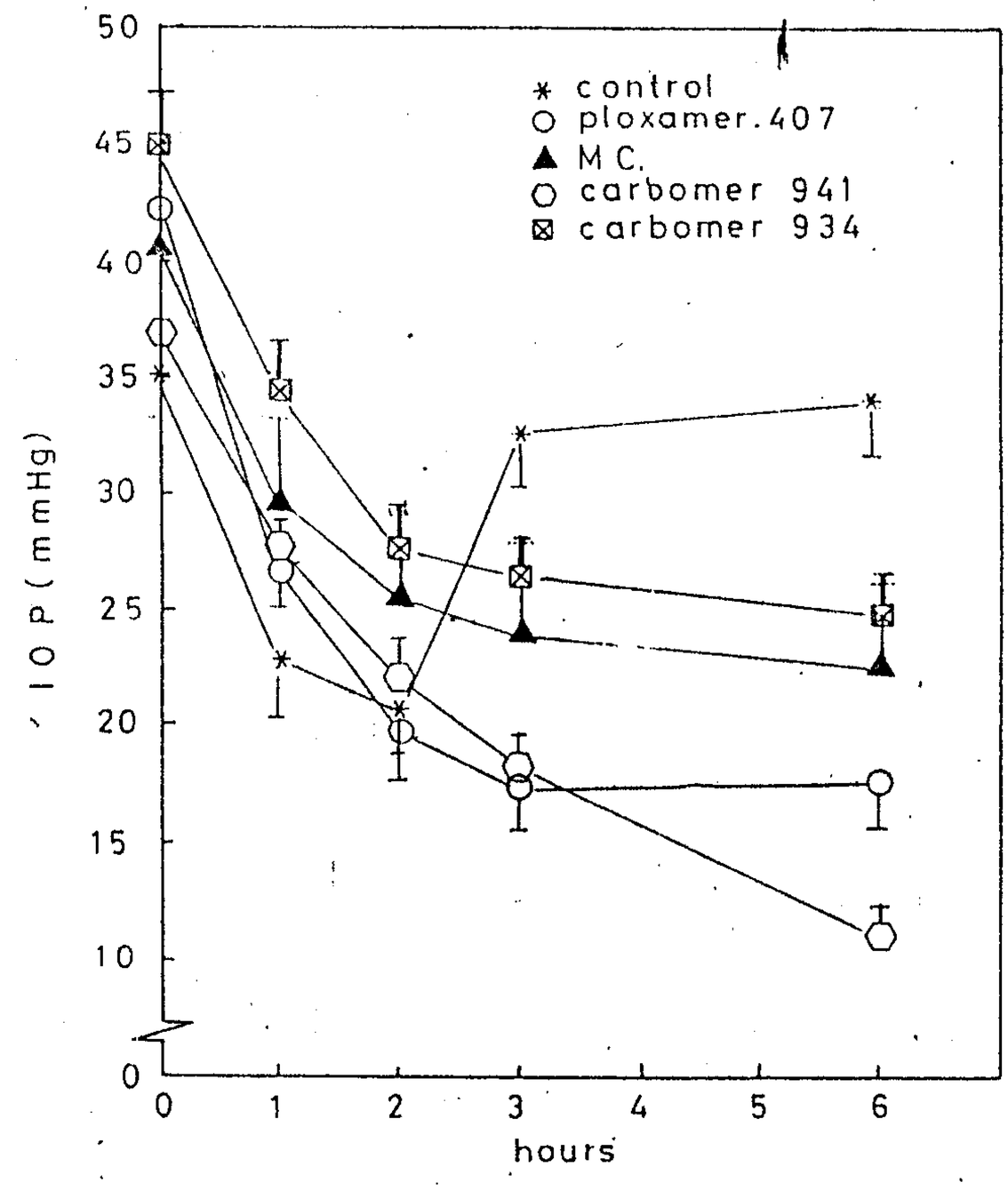


Fig. 1. Intraocular Pressure of Human's eye after application of 1.5 % w/w Carbachol Chloride Ophthalmic Gels.

ration of the drug into gel formulations is the sole factor which is responsible for the discussion of such results. Also, the data revealed that there is a correlation between the *in vitro* results and the *in vivo* ones. This is clearly appeared when the diffusion coefficient and the parameters of the drug activity were correlated. It should be noted that when the diffusion coefficient decreases the parameters of drug activity were found to be progressively increased.

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Bioavailability of Carbachol Chloride from Different Gel Formulations

التوافر الحيوى لعقار كلوريد الكاربا كول فى مستحضرات هلامية مختلفة

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تم تركيب مستحضرات هلامية مختلفة ذات خاصية الذوبان فى الماء لعقار كلوريد الكاربا كول وتمت دراسة انطلاق العقار من هذه المستحضرات وقد تم تقنين معامل الانتشار للعقار ووجد انه يتاثر كثيرا باللزوجة فى المحلول المائى - ووجد ايضا أن اللزوجة يكاد ينعدم تاثيرها فى المستحضرات الهلامية المختلفة. وايضا تم اجراء دراسة حيوية لمستحضرات العقار على المرضى الذين يعانون من الارتفاع الداخلى لضغط العين وقد وجد أن المستحضرات الهلامية للعقار لها تاثير خافض وطويل المدى لضغط العين الداخلى عند مقارنتها بالمحلول المائى لنفس العقار. وبينت هذه الدراسة على تقدير المساحة فوق المنحنى - والاستجابة القصوى - ووقت هذه الاستجابة - ايضا تم عمل المقارنات على اسس احصائية وتبين من الدراسة انه توجد علاقة ترابط بين النتائج المعملية ومثيلاتها على المرضى.

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