J. Pharm. Pharmacol. 1989, 41: 197–200
Communicated January 4, 1988

Sustained release ophthalmic formulations of pilocarpine

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Abstract—The bioavailability of drugs from conventional ophthalmic formulations is low. To optimize the therapy, sustained release ophthalmic dosage forms are warranted. Hydrogels such as sodiumcarboxymethyl cellulose, hydroxypropylmethyl cellulose, Carbopol-940, Carbopol-941 and Lutrol-FC-127 increase the duration of action of various drugs. Gels containing pilocarpine were prepared and evaluated by measuring the intensity and duration of miotic response in albino rabbits. Carbopol-940 gels, being the best of those used, were studied further for the effect of its concentration and of additives (benzalkonium chloride, phenylmercuric nitrate, chlorbutol and disodium edetate), autoclaving at 121°C for 30 min and irradiation with gamma rays (2·5 Mrad), on the end product.

Conventional ophthalmic formulations show lower bioavailability because of (i) constant lacrimal secretion and (ii) nasolacrimal drainage. This leads to frequent instillation of concentrated medication to achieve the desired therapeutic effect (Chien et al 1982). Systemic absorption of the drug drained through the nasolacrimal duct may result in some undesirable side effects (Schoenwald & Smolen 1971). Hence sustained release ophthalmic formulations are warranted. This can be achieved by prolonging the corneal contact time of medication (Chien et al 1982). Two major approaches investigated and practised in sustained release ophthalmic formulations are; (i) use of viscous gels (Schrenzel 1964; Giroux & Schrenzel 1964; Chrai & Robinson 1974; Tishina 1982; Miller & Donovan 1982; Neurnberg & Pruetting 1984; Habib et al 1985) and (ii) use of erodible or non-erodible inserts (Loucas & Haddad 1972; Shell & Baker 1974; Miyazaki et al 1982; Harwood & Schwartz 1982; Saettone et al 1984; Grass et al 1984; Hou et al 1985). Patented sustained release formulations of pilocarpine, Pilopine HS gel (Alcon 1985), Ocusert Pilo-20 and Ocusert Pilo 40 (Alza 1982), are available in the international market.

Sodiumcarboxymethyl cellulose (NaCMC), hydroxypropylmethyl cellulose (HPMC), Carbopol-940 (C-940) and Carbopol-941 (C-941) are known to give good gels in aqueous systems. Lutrol FC-127 (LFC-127) gives a solution at low temperatures but a good gel at 37°C. Hence it was thought worthwhile to investigate these polymers as sustained release materials for ophthalmic use. To achieve this goal, polymer gels containing pilocarpine nitrate (PN) (a cholinomimetic alkaloid salt used in the treatment of chronic simple glaucoma) were prepared and compared with conventional eye drops (obtained commercially) by measuring the intensity and duration of miotic response in albino rabbits.

Materials and methods

NaCMC (Cepol Dvp Spl. from Cellulose Products of India, Kathewar, Gujarat), HPMC (Methocel K-100M Premium from Colorcon, UK), C-940 and C-941 (Carbopol-940 and Carbopol-941 from BF Goodrich Chemical Company, USA) and LFC-127 (Lutrol FC-127 from BASF, West Germany) were used. Albino rabbits (Haffkine strain) were the test animals. The drug used was pilocarpine nitrate I.P.

Preparation of gels. NaCMC and HPMC were added to an aqueous solution of PN while stirring to give good gels. A dispersion of C-940 and C-941 in aqueous solution of PN when neutralized with sodium hydroxide solution (18% w/v) yielded a transparent gel. LFC-127 was dissolved in aqueous solution of PN at about 10°C. Gels prepared without the drug were used as controls.

Viscosity measurements. Apparent viscosities were measured using a Brookfield synchro-lectric viscometer model RVT with helipath assembly.

In-vivo studies. In-vivo studies were carried out in albino rabbits of either sex, 1·8–2·5 kg. These were kept in restraining boxes and were allowed to acclimatize to laboratory conditions for 1 h. The pupil diameter was measured with a vernier caliper held at 5 cm from the rabbit eye. Lighting was kept constant throughout the experiment. All experiments were carried out at room temperature (30°C). A minimum of four rabbits were used in each experiment. A minimum of two readings of pupil diameter were taken before administration of gels. The formulation (0·05 mL) was administered with the help of an insulin syringe in the lower cul-de-sac of one eye. The control (0·05 mL) was administered in the other eye. The pupil diameter was measured periodically until it regained its original size in most animals. The same animals were used repeatedly allowing a minimum of two days between two successive experiments.

Results and discussion

Viscosity measurement results are shown in Tables 1 and 2. Mean reduction in pupil diameter vs time was plotted. Fig. 1 shows a representative plot. For interpreting the miotic response results, one mm mean reduction in pupil diameter was considered a significant miotic response. The various miotic response parameters obtained from experiments in which different formulations were employed are summarized in Tables 3, 4.

Table 1. Viscosity of the gels.

	Viscosity (cP × 10^4)	
Gels	Plain	PN 2% w/v
NaCMC 4% w/v[a]	18·6	14·2
HPMC 4% w/v[b]	62·0	60·0
LFC-127 20% w/w[a]	14·6	12·4
C-940 1% w/v[a]	10·0	3·4
C-940 2% w/v[a]	11·6	9·6
C-940 4% w/v[a]	19·8	19·0
C-940 6% w/v[a]	27·2	26·4
C-941 6% w/v[a]	13·2	10·8

Brookfield synchro-lectric viscometer model RVT with helipath assembly at 5 rev min^{-1}. Spindles a = T-D, b = T-F.
Viscosity was measured at 37°C in case of LFC-127 gels and at room temperature (30°C) for all other gels. PN = Pilocarpine nitrate.

Effect of PN concentration. In the case of conventional eye drops, increasing the concentration of PN from 1 to 2% w/v caused a significant increase in the duration of miotic response (DR) (from 132 to 192 min). On the other hand, 4% w/v PN formulation gave a DR value (174 min) similar to that obtained

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Table 2. Effect of additives, autoclaving (121°C, 30 min) and gamma irradiation (2·5 Mrad) on viscosity of C-940 4% w/v gel.

Additive/process	Viscosity ($cP \times 10^4$) Without PN	With PN 2% w/v
Plain[x]	19·8	19·0
Phenyl mercuric nitrate[x] 0·004% w/v	20·4	19·4
Chlorbutol[x] 0·5% w/v	20·2	19·0
Benzalkonium chloride[x] 0·01% w/v	25·4	24·4
Disodium edetate[x] 0·1% w/v	21·2	18·6
Before autoclaving[x]	20·0	19·4
After autoclaving[x]	18·8	18·6
Before irradiation[y]	36·0	34·0
After irradiation[y]	57·6	64·0

Brookfield synchro-lectric viscometer model RVT with helipath assembly with spindle T–D; speed x = 5 rev min^{-1}, y = 2·5 rev min^{-1}. PN = Pilocarpine nitrate.

Viscosity was measured at room temperature (30°C) in all gels.

with 2% w/v formulation (Table 3). Hence formulations containing 2% w/v PN were prepared for further studies.

Effect of polymers. NaCMC (4% w/v), HPMC (4% w/v), LFC-127 (20% w/w), C-940 (2% w/v) and C-941 (6% w/v) gave gels with good consistency. Addition of PN caused a decrease in the viscosity of the gels and this effect was more pronounced in NaCMC 4% w/v and C-940 1% w/v gels (Table 1). Viscosities of NaCMC (4% w/v), HPMC (4% w/v) and LFC-127 (20% w/w) gels were higher than those of C-940 (2% w/v) and C-941 (6% w/v) gels (Table 1). Even then, NaCMC (4% w/v), HPMC (4% w/v) and LFC-127 (20% w/w) gels did not show significant increases in DR, whereas, C-940 (2% w/v) and C-941 (6% w/v) showed a significant increase in DR [From 192 to 246 min in the case of C-940 (2% w/v) and from 192 to 273 min in the case of C-941 (6% w/v)] (Fig. 1, Table 3). Hence it can be assumed that intrinsic properties of the polymer other than the viscosity are responsible for producing slower release. This may be because of in-situ salt formation between the polyacrylic acid of carbopol and pilocarpine (Saettone et al 1984; Grass et al 1984). C-940 gels were studied in detail because lower concentration of C-940 (2% w/v) gave DR (246 min) comparable to DR (273 min) obtained from a higher concentration of C-941 (6% w/v) (Table 3).

Effect of C-940 concentration. Higher concentrations of C-940 gave a substantial increase in DR (414 min in case of C-940 6% w/v) (Table 3). Gels containing 4% w/v C-940 were used for further studies because higher concentrations of C-940 gave very viscous gels and hence may not be pharmaceutically acceptable.

Effect of additivies. Benzalkonium chloride and phenyl mercuris nitrate did not cause a significant change in DR of C-940 gels (4% w/v) containing PN (2% w/v) (Table 4), whereas chlorbutol and disodium edetate reduced the DR (Table 4). Benzalkonium chloride caused a substantial increase in viscosity of C-940 gels (Table 2).

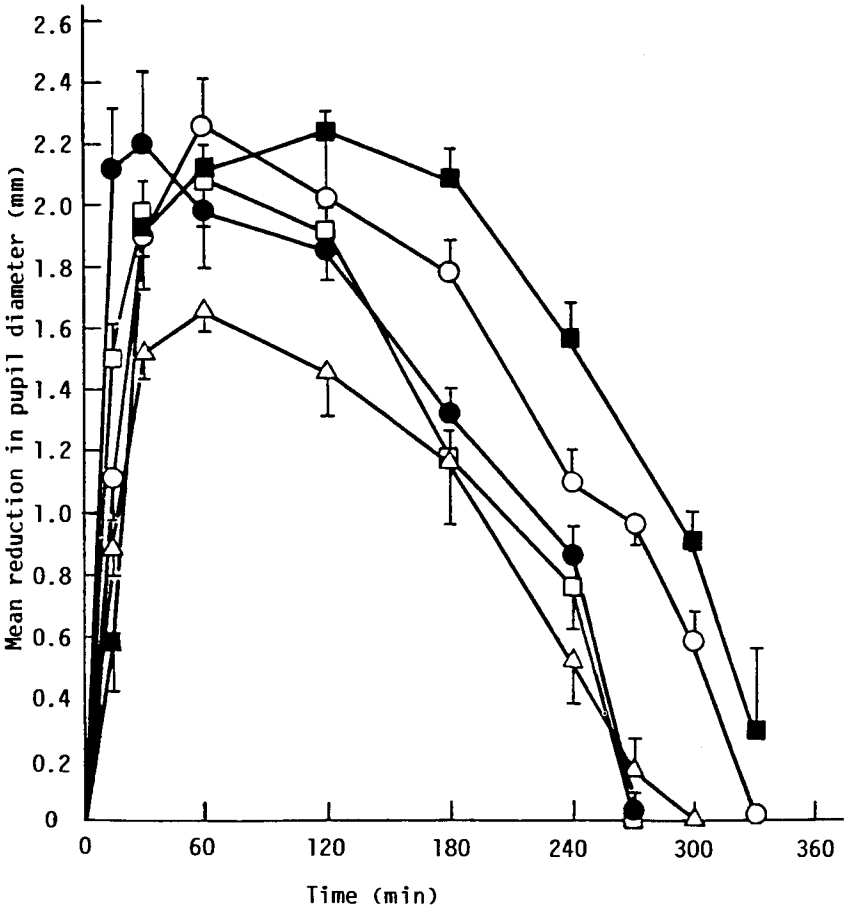

FIG. 1. Pilocarpine nitrate 2% w/v: effect of various polymers on miosis. (Vertical bar indicates standard error of mean $n \geq 4$.) —□— NaCMC (Cepol Dvp Spl.) 4% w/v, —○— Carbopol 940 2% w/v, —△— HPMC (Methocel K 100 M Premium) 4% w/v, —■— Carbopol 941 6% w/v, —●— Lutrol FC 127 20% w/w.

Table 3. Miotic response of PN formulations.

Preparation	PN concn % w/v	TM min	TP min	PR mm	DR min
Conventional eye drops	1	9	30	1·95±0·03	132
Conventional eye drops	2	9	60	2·24±0·20	192
Conventional eye drops	4	9	30	2·40±0·27	174
NaCMC 4% w/v gel	2	9	60	2·08±0·15	195
HPMC 4% w/v gel	2	18	60	1·65±0·05	177
LFC-127 20% w/w gel	2	6	30	2·2±0·23	216
C-940 1% w/v gel	2	12	30	1·93±0·05	237
C-940 2% w/v gel	2	12	60	2·25±0·17	246
C-940 4% w/v gel	2	12	120	1·9±0·16	321
C-940 6% w/v gel	2	21	180	2·05±0·1	414
C-941 6% w/v gel	2	18	120	2·24±0·06	273

TM: Time required to achieve a significant miotic response (1 mm mean reduction in pupil diameter).
TP: Time required to achieve peak miotic response.
PR: Peak miotic response ± s.e.m. $n \geq 4$.
DR: Duration of significant miotic response.
PN: Pilocarpine nitrate. 0·05 mL of the formulation was administered in the lower cul-de-sac of the rabbit eye.

Table 4. Effect of additives, autoclaving (121°C, 30 min) and gamma irradiation (2·5 Mrad) on miotic response of C-940 4% w/v gels containing 2% w/v PN.

Preparation	TM min	TP min	PR mm	DR min
Plain	12	120	1·9±0·16	321
With phenyl mercuric nitrate 0·004% w/v	18	60	2·0±0·14	312
With chlorbutol 0·5% w/v	15	60	2·06±0·13	267
With benzalkonium chloride 0·01% w/v	21	60	2·06±0·04	309
With disodium edetate 0·1% w/v	27	60	2·06±0·14	279
Plain gel after autoclaving	21	60	1·82±0·11	312
Plain gel after irradiation	21	60	2·18±0·12	297

TM: Time required to achieve a significant miotic response (1 mm mean reduction in pupil diameter).
TP: Time required to achieve peak response.
PR: Peak miotic response ± s.e.m. $n \geq 4$.
DR: Duration of significant miotic response. 0·05 mL of the gel was administered in the lower cul-de-sac of the rabbit eye.

Effect of autoclaving and irradiation. Autoclaving at 121°C for 30 min and irradiation with gamma rays (2·5 Mrad) did not cause a significant change in DR of C-940 (4% w/v) gel containing PN (2% w/v) (Table 4). Autoclaving caused marginal change in viscosity whereas irradiation increased viscosity substantially (Table 2). Autoclaving did not affect the consistency of the gel, but irradiation gave a brittle gel. Hence autoclaving appears to be the better method of sterilization.

The time required to achieve a significant miotic response (TM) was below 30 min in all formulations with a minimum of 6 min in the case of LFC-127 20% w/w gel containing 2% w/v PN (Table 3) and a maximum of 27 min in the case of C-940 4% w/v gel containing 2% w/v PN and 0·1% w/v disodium edetate (Table 4). The lower TM with LFC-127 gel can be attributed to the surface active property of LFC-127, whereas the higher TM with C-940 gel can be attributed to the tendency of C-940 to sustain the action. The time required to achieve peak response varied between 30 min and 180 min. The gradual increase from 30 min (for C-940 1% w/v gel) to 180 min (for C-940 6% w/v gel) is interesting and indicates a sustained-release property of C-940 gels (Table 3). The peak response varied from 1·65 mm ± 0·05 (for HPMC 4% w/v gel containing 2% w/v PN) to 2·40 mm ± 0·27 (for conventional eye drops containing 4% w/v PN) (Table 3).

Conclusions

The present investigation clearly indicates the superiority of C-940 as a sustaining agent for pilocarpine. Interaction of C-940 and benzalkonium chloride needs further study. C-941 in a higher concentration may give a good sustained release formulation of pilocarpine.

The authors thank the University Grants Commission, India; Dr M. A. Shenoy of U.D.C.T. Bombay; Mr S. R. Khanna of FDC Private Ltd, Bombay; Mr B. S. Barve and Mr S. M. Shanbhag of Lakme Ltd, Bombay; Theresa Gyorok of BF Goodrich Chemical Company, USA; Mr Dori Schmetterling of Colorcon, UK; Dr B. K. Joshi of Grant Medical College, Bombay; Cellulose Products of India, Gujarat; Maharukh Patel of Burroughs Wellcome Ltd, Bombay; Dr B. P. Chandrasekhar of BASF, Bombay and Dr S. Dayal of Pfizer Ltd, Bombay, for their timely help during the progress of this work. The authors also thank Dr R. V. S. V. Vadlamudi of U.D.C.T. Bombay and Dr M. W. Dikshit of E. Merck, Bombay, for their valuable suggestions during the progress of this work.

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J. Pharm. Pharmacol. 1989, 41: 200–202
Communicated June 24, 1988

Transport characteristics of [^{3}H]-chlorpromazine across rat small intestinal brush border membrane

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Abstract—The transport mechanism of chlorpromazine, a tertiary amine, has been investigated using brush border membrane vesicles isolated from rat small intestine. Chlorpromazine was taken up rapidly by the vesicles the uptake being mainly due to binding to the membrane. The transport of chlorpromazine into the intravesicular space was facilitated by the transmembrane electrical potential difference (inside negative) induced by valinomycin or sodium thiocyanate. This facilitating effect was observed only when the transmembrane electrical potential difference was induced after chlorpromazine uptake had reached a steady state. In the initial phase of chlorpromazine uptake, there was no effect. Therefore, it is suggested that both rapid binding to brush border membrane and transmembrane electrical potential difference (inside negative) across the membrane plays a significant role in the transport processes of chlorpromazine through the intestinal epithelium.

Chlorpromazine, a tertiary amine compound (TAC) used orally as a potent major tranquilizer, is a cationic amphiphilic drug possessing both a hydrophobic side chain and a hydrophilic residue in its structure. It has been shown that chlorpromazine binds to liposomes and biological membranes such as erythrocytes (Sheetz & Singer 1974; Zachowski & Durand 1988). Although it is mostly ionized over the pH range in the gastrointestinal tract (Green 1967), it is well known that its absorption is rapid (Curry et al 1971). This phenomenon is common for other TACs such as promethazine, imipramine and diphenhydramine. But there are few reports on the transport mechanism of these TACs across brush border membrane.

In our previous reports dealing with the transport mechanism of quaternary ammonium compounds (QACs) across the brush border membrane isolated from rat small intestine, we have shown that there might be at least two processes in the transport of propantheline, an anti-acetylcholine QAC, across the membrane (Saitoh et al 1987, 1988a). Initially, propantheline binds rapidly to brush border membrane and then enters into the epithelium stimulated by the transmembrane electrical potential difference (inside negative). Moreover, we have indicated that the binding of propantheline to the brush border membrane is competitively inhibited by several TACs such as chlorpromazine. Therefore, it is suggested that there is a common transport mechanism across brush border membrane between TACs and QACs.

As one approach to clarify the absorption mechanism of TACs, we have examined the transport characteristics of chlorpromazine across the brush border membrane.

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Materials and methods

Chlorpromazine hydrochloride and valinomycin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [^{3}H]-Chlorpromazine hydrochloride (27·1 Ci mmol^{-1}) was obtained from New England Nuclear Co. (Boston, MA, USA). All other chemicals were of the highest grade available commercially and were used without further purifications.

Brush border membrane vesicles were isolated from the entire small intestine of male Wistar rats (250-300 g) according to the calcium chloride precipitation technique of Kessler et al (1978). The purity of the membrane was routinely evaluated by the enrichment of alkaline phosphatase (E.C.3.1.3.1.), an enzyme