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COMMUNICATION

Ophthalmic Vehicles Containing Polymer-Solubilized Tropicamide: "In Vitro/In Vivo" Evaluation

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ABSTRACT

Commercial 1.0% aqueous tropicamide (TR) eyedrops are buffered to pH 4.4–5.0 to produce sufficiently stable solutions of the weakly basic, poorly soluble drug. These acidic solutions, however, are irritants and may induce copious lachrimation, thus reducing the drug bioavailability. The aim of the present study was to evaluate some solubilizing agents for the preparation of 1.0% TR ophthalmic solutions adjusted at physiologically compatible pH, potentially showing increased eye tolerance, activity, and stability when compared with standard commercial eyedrops. The tested solubilizers were two non-ionic surfactants—Tyloxapol (TY) and Cremophor EL (CR)—and one polymer, Pluronic P85 (PL). Four stable 1% TR formulations, containing 3% TY, 7.5% CR, 15% PL, or 5% CR + 10% PL were submitted to mydriatic activity tests in rabbits. They improved to a small but statistically significant extent the AUC for mydriatic effect of TR in the test animals when compared with commercial 1.0% TR eyedrops.

Key Words: *Tropicamide; Tyloxapol; Cremophor EL; Pluronic P85; Solubilization; Mydriatic effect; Rabbits*

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INTRODUCTION

The relatively low water solubility (0.56–0.57% w/v at 25°C) of tropicamide (TR), a synthetic mydriatic and cycloplegic drug, can be increased by adjusting the pH of the solution to a relatively low value (≤ 5.0), to increase the ionization and hence the solubility of the weak base. The TR solubility in 1.0% w/v "acidic" commercial eyedrops routinely used for refractive examination, however, critically depends on pH, storage temperature, and ion content of the solution. Furthermore, the ocular availability of TR administered with these collyria is poor, since their low pH elicits an irritant response, resulting in lachrimation and quick elimination from the precorneal area. These facts have stimulated in time the development of alternative, more physiological TR ophthalmic formulations.

A previous report from our laboratory (1) dealt with solubilization of TR by a series of poloxamers (Pluronics). A study on liposomal TR formulations was published in 1999 by other authors (2). In a recent investigation (3), we investigated the solubilization of TR by hydroxypropyl-β-cyclodextrin and water-soluble polymers. The aim of the present study was to evaluate a further series of agents for the preparation of 1.0% TR ophthalmic solutions adjusted at a physiologically compatible pH, potentially showing increased eye tolerance, activity, and stability when compared with standard commercial eyedrops.

Two non-ionic surfactants, Tyloxapol (TY) and Cremophor EL (CR), and one polymer, Pluronic P85 (PL), were tested in the present study as solubilizers for TR. The resulting 1% formulations were submitted to stability tests and to mydriatic activity tests in rabbits, using commercial 1% TR eyedrops as reference.

EXPERIMENTAL

Materials

TR was obtained from TCI (Tokyo, Japan); Cremophor EL (CR) and Pluronic P85 (PL) from BASF AG (Ludwigshafen A/R, Germany); and Tyloxapol (TY) from SIGMA (St. Louis, MO). All other chemicals, solvents, etc., were of analytical grade.

TR Solubility Tests

Solutions of TY, CR, and PL at different concentration (from 1.0 to 20.0% w/v) in distilled water containing excess TR (3.0% w/v) were shaken for 24 hr in a thermostated water bath (25°C), and then filtered through a 0.2-μm membrane filter (Millipore Millex®—GS). The drug content was determined spectrophotometrically (253 nm) against blank solutions containing the appropriate amount of each polymer, after dilution with CH_3CN (1:200) and filtration (0.2-μm membrane filter, same type as specified above). The spectrophotometric method of analysis for TR—due to the absence of interfering UV-absorbing substances and to its high sensitivity—was found to be satisfactory.

Viscosity Measurements

Since the polymeric solutions showed a Newtonian type of flow, the viscosity relative to water (η_{rel}) of the test solutions and of the commercial reference eyedrops was determined by capillary viscometry at 20°C (Canon-Fenske series 75 viscometer); the relevant density values were determined using a hydrostatic balance (Sartorius, mod. 6080/60801).

Ophthalmic Formulations

Four test solutions (3.0% w/v TY, 7.5% w/v CR, 15% w/v PL, and 5% w/v CR + 10% w/v PL, all containing 1.0% w/v TR and 0.01% w/v benzalkonium chloride as preservative) were made appropriately isotonic with NaCl (Roebling Osmometer) and then filtered through 0.2-μm filters prior to use. pH measurements (Orion pHmeter) were performed immediately after preparation; no substantial changes were observed after 18 months storage at room temperature (19–21°C). The pH, osmolality, and relative viscosity values of the reference and test solutions are listed in Table 1.

Mydriatic Activity Tests

The tests on the four TR vehicles were carried out on male, non-anesthetized New Zealand albino rabbits (2.0–2.5 kg, Pampaloni Rabbitry, Fauglia, Italy), selected on the basis of their similar response to light intensity and to the mydriatic activity of TR. The animals were used and treated as prescribed in

Table 1

pH and Osmolality Values of 1% TR Ophthalmic Solutions

Formulation	pH	Osmolality (mOsmol/kg)	$Viscosity_{rel}$
1. RS[a]	4.9	~300	1.08
2. TY 3%	7.0	~290	1.31
3. CR 7.5%	6.0	~300	5.98
4. PL 15%	7.2	~300	5.40
5. CR 5% + PL 10%	6.2	~300	7.49

[a]Reference solution: commercial 1.0% TR eyedrops.

the publication "Guide for the care and use of laboratory animals" (NIH Publication No. 92–93, revised 1985). The animals were allowed to move their heads freely, and their eye movements were not restricted. The overall procedure and experimental details suggested by Smolen and Schoenwald (4) and also reported in a previous paper (5) were followed throughout.

Each solution was tested on groups of at least nine rabbits; the administered dose was 10 µl in all cases. Commercial 1.0% aqueous TR eyedrops (Visumidriatic 1%, Visufarma s.r.l., Rome, Italy) were used as reference.

RESULTS AND DISCUSSION

The solubility of TR increased ~1.9 times, ~2.3 times, and ~2.6 times with respect to the water solubility at the same temperature (0.56 g/100 ml), in the presence of 5.0% w/v TY, 12.5% w/v CR, and 20.0% w/v PL, respectively. The solubility isotherms of TR at 25°C in different concentrations of CR, PL, and TY (% w/v TR solubilized vs. concentration of solubilizer, also expressed in % w/v) are illustrated in Fig. 1. As shown in the figure, the saturation solubility of the drug increased almost linearly with increasing solubilizer concentration in the ranges 1.0–3.5% w/v for TY, 1.0–12.5% w/v for CR, and 1.0–20.0% w/v for PL. In the case of TY, the saturation solubility of TR showed a maximum (plateau) value in the TY concentration range 5–10%, then decreased with a further increase in surfactant concentration.

On the basis of these results, the four ophthalmic solutions (2–5) containing 1.0% w/v TR listed in Table 1 were submitted to biological tests. These

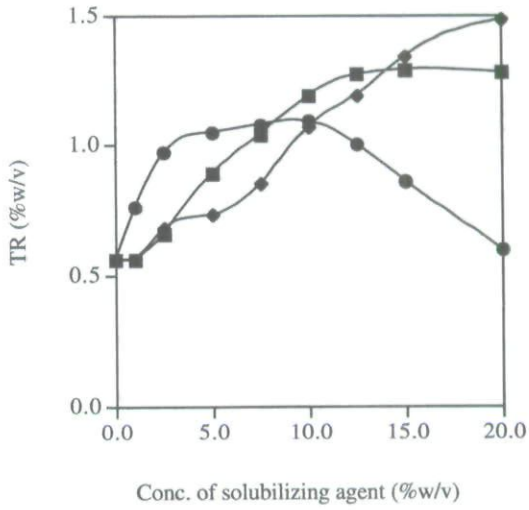

Figure 1. Solubility isotherms of tropicamide in the presence of different agents at 25°C: ●, TY; ■, CR; ◆, PL.

did not show TR crystals after 18 months' storage at room temperature.

The results of the mydriatic activity tests on albino rabbits, summarized in Table 2, indicate that the four solutions, containing 3% TY (2), 7.5% CR (3), 15% PL (4), and 5% CR + 10% PL (5), improved to a small but statistically significant extent the mydriatic AUC of TR when compared with the commercial reference solutions, RS.

The activity increases of TR formulation 2, 3, and 4 were associated with an increased duration (about 60 min) of the mydriatic effect and with an increased I_{max} (maximal mydriatic response) value, even if not to a statistically significant degree.

The improved effect of formulations 2–4 with respect to the reference solution, RS, is in all evidence to be attributed to their more "physiological" pH value (7.0 for TY, 6.0 for CR, 7.2 for PL, vs. 4.9 for RS), resulting in reduced irritation and lachrimation, and hence in prolonged retention at the absorption site. The influence of viscosity effects on ocular retention is probably very low, since a 6-fold increase in relative viscosity (formulation 6 vs. 2) did not correspond to similar increase in relative AUC. As, also indicated by Patton and Robinson (6), in a study of retention of viscous solutions in rabbit eyes, within the relatively narrow viscosity range of the present vehicles, changes in drainage loss from the eye (and hence, of bioavailability) should be very modest.

Table 2

Summary of the Activity Parameters in Rabbits of TR 1% in Ophthalmic Solutions

Formulation TR 1.0%	Peak Time (min)	I_{max} (mm) (±SEM)	Duration (min)	AUC ($cm^2 \pm SEM$)	AUC_{rel}
1. RS[a]	90	2.278 ± 0.09	360	131.9 ± 3.23	1.00
2. TY 3%	60	2.500 ± 0.08	420	149.8 ± 5.84*	1.13
3. CR 7.5%	180	2.333 ± 0.08	420	153.3 ± 5.54*	1.16
4. PL 15%	60	2.500 ± 0.00	420	150.8 ± 5.30*	1.14
5. CR 5% + PL 10%	90	2.833 ± 0.08*	360	148.4 ± 5.92*	1.12

[a]Reference solution: commercial 1.0% TR eyedrops, pH 4.9.
*Significantly different from the RS value ($p < 0.05$, Student's *t*-test).

Among the tested solubilizers, TY and PL reduced the peak time from 90 (RS) to 60 min, while CR delayed the peak time to 180 min. It might be speculated that the reduction of the time to peak shown by TY and PL with respect to the reference solution might be attributed to a lower irritant effect, resulting in decreased lachrimation and tear dilution. Conversely, TR binding (or micellization) by CR, and a consequently slower release, might be responsible for the peak time delay observed with this formulation.

The AUC increase of formulation 5 (5% CR + 10% PL) was essentially determined by a significantly increased I_{max}, whereas both the duration of mydriatic effect and peak time remained unchanged with respect to the reference solution. This effect might be attributed to a prolonged retention at the absorption site, possibly due to the slightly higher viscosity (7.49 vs. 1.08 for RS) and pH values (6.2 vs. 4.9 for RS) of this formulation.

Some information on these solubilizers might be of relevance here. TY, described as a nonionic liquid surfactant of the alkyl-aryl-polyether alcohol type in the official monographs of U.S.P. XXVI, is endowed with detergent-solubilizing properties (7) and is widely used as pharmaceutical additive. Its solubilizing and micelle-forming properties have been described (8,9). Cremophor EL (CR), the brand name of polyoxyl-35 castor oil, is a mixture of the triricinoleate ester of ethoxylated glycerol with smaller amounts of macrogol ricinoleate and the corresponding free glycols (10). This surfactant is an emulsifying and solubilizing agent (10) widely used as adjuvant-solubilizer in parenteral (11) and, at the 5% concentration, in topical ophthalmic formulations (12). Pluronic P85 (PL) belongs to the series of polyoxyethylene–polyoxypropylene (POE/POP) block copolymers named poloxamers or pluronics. Applications of these agents in the ophthalmic field have been recently reported (13,14).

In conclusion, Tyloxapol (TY), Cremophor EL (CR), and Pluronic P85 (PL) proved safe and effective solubilizers for TR, producing 1.0% solutions without any crystallization problem after 18 months storage at room temperature. The solutions were well-tolerated in rabbits, and proved more efficient with respect to standard commercial "acidic" eyedrops. The present preliminary results, which of course cannot be extrapolated to humans, will be implemented with further pharmacokinetic studies in rabbits.

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REFERENCES

1. Saettone, M.F.; Giannaccini, B.; Delmonte, G.; Campigli, V.; Tota, G.; La Marca, F. Int. J. Pharm. **1988,** *43*, 67.
2. Nagarsenker, M.S.; Londhe, V.I.; Nadkarni, G.D. Int. J. Pharm. **1999**, *190*, 62.
3. Cappello, B.; Carmignani, C.; Iervolino, M.; La Rotonda, M.I.; Saettone, M.F. Int. J. Pharm. **2001**, *213*, 75.
4. Smolen, V.F.; Schoenwald, R.D. J. Pharm. Sci. **1974**, *63*, 1582.
5. Saettone, M.F.; Giannaccini, B.; Barattini, F.; Tellini, N. Pharm. Acta Helv. **1982**, *47*, 3.
6. Patton, T.F.; Robinson, J.R. J. Pharm. Sci. **1975**, *64*, 1312.

7. Lewis, A.J. *Modern Drug Encyclopedia*, 16th edn. Yorke Medical Books: New York, 1981.
8. Guttman, D.E.; Hamlin, W.E.; Shell, J.W.; Wagner, J.G. J. Pharm. Sci. **1961**, *50*, 305.
9. Regev, O.; Zana, R. J. Colloid Interface Sci. **1999**, *210*, 8.
10. *Martindale: The Complete Drug Reference*, Parfitt K. Ed.; 32nd edn. The Pharmaceutical Press: London, 1999; 1327.
11. Van Zuylen, L.; Gianni, L.; Verweij, J. et al. Anticancer Drugs **2000**, *11*, 331.
12. Reer, O.; Bock, T.K.; Müller, B.W. J. Pharm. Sci. **1994**, *83*, 1345.
13. Bochot, A.; Fattal, E.; Gulik, A.; Couarraze, G.; Couvreur, P. Pharm. Res. **1998**, *15*, 1364.
14. Desai, S.D.; Blanchard, J. J. Pharm. Sci. **1998**, *87*, 1190.