# The effect of different ophthalmic vehicles on the activity of tropicamide in man

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The most common vehicles for topical ophthalmic drugs are 'regular' saline solutions, viscous saline solutions and semisolid paraffin ointments. Semisolid aqueous gels are seldom used clinically, in spite of evidence of greatly enhanced therapeutic responses in the rabbit (Giroux & Schrenzel 1964; Bottari et al 1979). Whether viscous saline solutions are superior to 'regular' ones in increasing the drug bioavailability in man has been occasionally raised (Linn & Jones 1968; Mattila et al 1968; Waltman & Patrowicz 1970; Adler et al 1971; Trueblood et al 1975; Melis-Decerf & Van Ooteghem 1979) and is still apparently unsettled. The present study was undertaken to evaluate critically on human subjects (a) the effect of a viscous vs a 'regular' solution, and (b) the effect of an aqueous gel vs a viscous solution and an ointment. Vehicles containing three increasing concentrations (0·05, 0·1 and 0·2% w/v) of tropicamide were used.

Commercial tropicamide (Prodotti Roche) was purified to constant m.p. 97–99 °C. A sample was micronized (Fryma JMRS–80 jet mill) to yield particles of average diameter (geom., microscopic analysis) 4·5 μm. The 'regular' aqueous saline solutions (AS) were prepared with 0·2 M, pH 7·0 Sørensen phosphate buffer, whose tonicity was adjusted with NaCl. The same vehicle, containing 0·7% w/v hydroxypropylcellulose (Alcogel, Lab. Vevy) was used for the preparation of the viscous solutions (VS). The aqueous gels (AG) were prepared by neutralizing with diisopropanolamine (Fluka A.G.) 0·3% w/v dispersions of carboxyvinyl polymer (Carbopol 940, B.F. Goodrich Chemical Co.) containing the appropriate amount of tropicamide. All of these preparations were autoclaved at 2 bars for 20 min. The pH after sterilization was in the range 6·4–7·1. The ointment vehicle (OV) was a 70:30 w/w mixture of yellow soft paraffin and liquid paraffin (both B.P. grade, Carlo Erba S.p.A.). Micronized tropicamide was incorporated into this vehicle by levigation under sterile conditions: no final sterilization was carried out.

The preparations were tested on 210 Caucasian volunteers of either sex, aged 15–65 years: subjects with conjunctival or corneal abrasions and disorders, or glaucoma, were excluded. Each concentration was tested on groups of at least 20 subjects. The horizontal diameter of the pupil was estimated to the nearest 0·1 mm with a micrometer held always at the same distance from the subjects' face, by the same operator.

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All measurements were made in the same room, in which the level of illumination was held rigorously constant throughout the study. The applied dose was 50 μl. The semisolid preparations were applied to the partially everted lower lids; after instillation or application the subjects were instructed to keep their eyes closed for 35–40 s to avoid reflex blinking and/or lacrimation. Both eyes were treated, and a single value was considered for each subject since the responses were in all cases perfectly symmetrical in the two sides.

Fig. 1 shows the results observed with the four

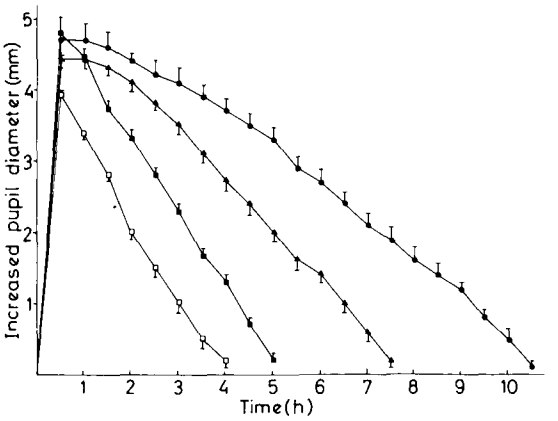


FIG. 1. Mydriatic effect of the preparations containing 0·2% w/v tropicamide. ● AG; ▲ OV; ■ VS; □ AS. Vertical lines indicate ± 1 s.e.m.

vehicles containing 0·2% w/v tropicamide: the data are presented as the average increased pupil diameter, in mm ± s.e., over the initial mean value of each group. Analogous response-time patterns, and relative orders of activity were observed with the vehicles containing 0·05 and 0·1% tropicamide. The peak time (time of maximum change in pupillary diameter) was ca 30 min for all preparations; the activity decrease was immediate for AS and VS, while it was somewhat delayed (ca 1 h) for AG and OV, irrespective of the drug concentration. The semisolid vehicles, AG and OV, showed, particularly at the 0·2% concentration, a sustained activity (cf. Fig. 1) even if the true plateau region was rather limited. Statistical differences in the mydriatic activity induced by increasing concentrations of the drug in the vehicles can be easily assessed from the graphs in Fig. 2ab. In the Figure the activity (increased pupil

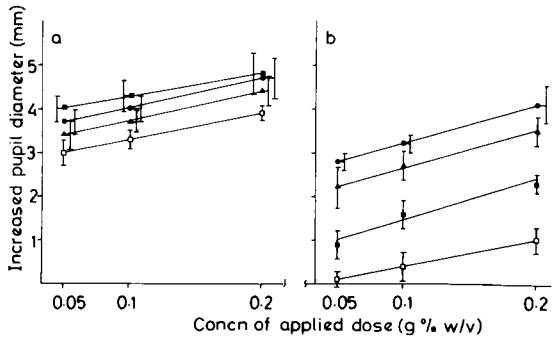


FIG. 2. Dose-effect relationships of the four preparations at the peak time (30 min) and at 180 min (a and b, respectively). ● AG; ▲ OV; ■ VS; □ AS. Vertical lines indicate 95% confidence limits: some lines have been displaced sideways for clarity.

diameter in mm with the associated 95% confidence limits) at two selected times (peak time and 180 min) is reported on the ordinate vs the tropicamide concentration in the applied doses. At the peak time (30 min, Fig. 2a) the peak mydriatic intensities ($I_{max}$) elicited by VS, OV and AG were not statistically different (5% probability level) from each other. However, the $I_{max}$ induced by the viscous solution, VS, and by the aqueous gel, AG, showed a small but statistically significant increase over that produced by AS. The sustaining effect of the semisolid preparations is evidenced in Fig. 2b: after 180 min, while the activity of AS was greatly decreased, or terminated at the lowest concentration, the activity induced by AG and OV was still appreciable, particularly at the 0·2% concentration. The responses elicited after 180 min by the two semisolid preparations were not statistically different from each other. However, at all further times the mydriatic activity mediated by AG was significantly greater than that of OV (cf. Fig. 1). In Fig. 3 the areas under the mydriasis-time curves (AUC) are reported for each vehicle vs the concentration of the drug in the applied doses. The areas should reflect the aqueous humour concentration of tropicamide (Chrai & Robinson 1974), thus offering a quantitative estimate of the drug availability from the various vehicles. For all vehicles, the AUC increased linearly with increasing drug concentration, thus excluding site saturation during the transcorneal transport within the presently examined concentration range (cf. Krohn & Breitfeller 1979). At the 0·2% concentration the relative AUC of the vehicles, respect to the AUC of AS considered equal to 1, were 1·63 for VS, 2·61 for OV and 4·01 for AG. As shown in Fig. 3, these values were all significantly different ($P < 0.05$) from each other.

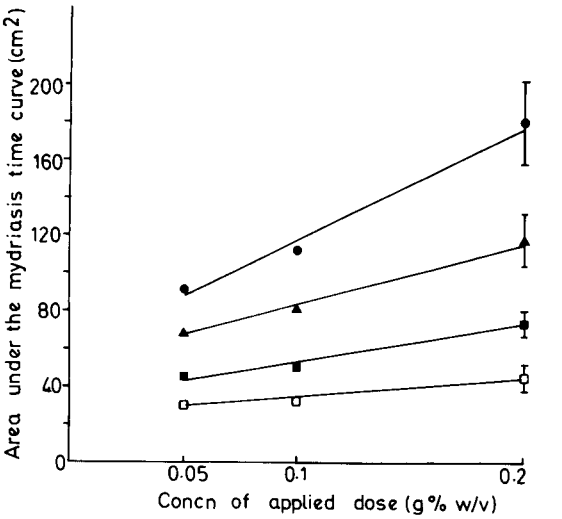


FIG. 3. Areas under the mydriasis-time curves vs concentration of the applied doses. ● AG; ▲ OV; ■ VS; □ AS. Vertical lines indicate 95% confidence limits. The areas were calculated from graphs where units are mm $h^{-1}$. On the vertical axis 3·0 cm corresponded to 1·0 mm of pupil diameter increase; on the horizontal axis 1·0 cm corresponded to 0·5 h.

The observed moderate increase (or no increase, in the case of OV) of $I_{max}$, without any concomitant change in the peak time would rule out for VS, OV and AG vehicle controlled release and/or vehicle-mediated transport, and point rather to improved ocular retention as the reason for the increased tropicamide bioavailability (cf. Schoenwald et al 1978; Krohn & Breitfeller 1979). For ointment-type vehicles, improved retention results also from previous literature data (Hardberger et al 1975; Sieg & Robinson 1975). In the case of OV, a slow dissolution rate of the suspended drug particles, associated with a poor tear-miscibility of the paraffin mixture probably reduced the positive effects resulting from a prolonged contact time.

Ocular contact time has often been related to vehicle viscosity, and much effort has been devoted to the establishment of viscosity-retention time-biological response relationships. Although no such relationships will be discussed here, it seems reasonable to indicate viscosity effects as the cause of the increased bioavailability observed in the present cases. Rheograms of VS, OV and AG, measured at 33 °C on a Rheomat 30 rotary viscometer (Contraves A.G.), indicate for these vehicles a pseudoplastic, non-thixotropic type of flow. If single-point viscosity data (i.e., data obtained at a single rate of shear) are considered, the apparent viscosity of the present vehicles increases in the order AS < VS < OV < AG.

In conclusion, a definite influence of the vehicles on the human response to tropicamide is apparent from this study. Statistically significant bioavailability increases were observed (a) for a viscous vs a 'regular' solution, and (b) for an aqueous gel vs other commonly used vehicles. The present data were obtained with tropicamide, but it is not unreasonable to expect analogous results with other ophthalmic drugs. The observation that eye dilations quite adequate for eye

examination could be obtained with tropicamide concentrations much lower (10–20 times) than those currently used in the clinical practice is also noteworthy. The use of dilute (0·05–0·1 % w/v) tropicamide solutions in suitable vehicles should be recommended in order to prevent potential risks of side effects and of systemic toxicity (cf. also Wang & Hammarlund 1970, Brown & Hanna 1978).

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# LETTERS TO THE EDITOR

## 2-Amino-6,7-dihydroxytetrahydronaphthalene and the receptor-site preferred conformation of dopamine—a commentary

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The study of the preferred conformation of neurotransmitters, hormones, peptides and drugs is an important one because of the possible usefulness of such information as an aid to the understanding of their molecular modes of action and at a practical level in the design of new agonists or antagonists (Richards 1977). The problem of preferred conformation is, however, twofold. It is possible to refer to the crystal, solution or vacuum preferred conformation of a molecule, depending on the method of conformational analysis, but with a very flexible molecule that this corresponds to the receptor-preferred conformation is not a certainty. Indeed, there is direct evidence that often these two preferred conformations are different (Burgen et al 1975; Williams 1977). One approach that avoids some of these difficulties is that of the rigid or semi-rigid analogue method (Portoghese 1970; Horn & Rodgers 1977). Here attempts are made to constrain the molecule into a hypothesized receptor-preferred conformation by, for example, the addition of an extra ring system to replace a flexible side chain. If activity is still retained, then depending on the rigidity of the analogue, it is possible to make inferences about the actual receptor site preferred conformation of the original non-rigid molecule.

Recently various attempts have been made to determine the receptor site preferred conformation of the neurotransmitter dopamine (DA) (Fig. 1a) (Dandiya et al 1975; Grol & Rollema 1977; Miller 1978). This process has been aided by the fact that various catechol derivatives of 2-aminotetrahydronaphthalene (ATN), which can be considered as DA analogues with a restricted conformation, have been shown to be very potent DA receptor agonists (Woodruff et al 1974, 1977; Miller et al 1974; McDermed et al 1975; Costall et al 1977). We now report details of the conformation in the crystal of one of these analogues namely 2-amino-6,7-dihydroxytetrahydronaphthalene (6,7-diOHATN) (ADTN or 6,7-dihydroxy-2-aminotetralin) (Figs. 1b and 3b) which enables us for the first time to suggest, with some degree of molecular detail (Table 1) and certainty, the most probable receptor conformation of DA.

The conformational analysis of the receptor preferred conformation of DA is a three-fold problem due to the following possibilities:

1. DA can exist in a *trans* or two *gauche* forms (Fig. 2a).
2. There are two possibilities for the *trans* form of DA (Fig. 2b) i.e. the catechol ring is perpendicular to the $-CH_2-NH_2$ bond (*trans* $\alpha$) or coplanar with it (*trans* $\beta$).
3. If the catechol ring is coplanar to the side chain (*trans* $\beta$) there are two further possibilities depending on the orientation of the catechol ring i.e. the $\alpha$ and $\beta$ rotamers (Fig. 2c).

Regarding the possibilities under section 1, some theoretical studies indicate a preference for the *trans* and some for the *gauche* form (Bustard & Egan 1971; Rekker et al 1972; Kier 1973; Pullman et al 1972,

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