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THE INFLUENCE OF THE DROPSIZE ON THE ELIMINATION OF AN OPHTHALMIC SOLUTION FROM THE PRECORNEAL AREA OF HUMAN EYES

A. Ludwig and M. Van Ooteghem

Dept. Pharmaceutical Sciences, University of Antwerp (UIA)
Universiteitsplein 1, B-2610 Wilrijk, Belgium.

ABSTRACT

In order to study the influence of the dropsize on the ocular contact time and on the drainage of a fluorescent tracer a non-invasive method, using a slit lamp fluorophotometer is employed. Quantitative measurements of the fluorescence decay in the tear film, after instillation of an iso-osmotic tracer solution ranging in volume from 20 to 1 µl, are performed.

In most cases an initial fast decay is followed by a slower elimination after a few minutes. The initial phase of the decay profile obeyes fairly well first order kinetics and the tear elimination coefficient is calculated. Important intra and inter subject variations are noted.

In contrast to results obtained with rabbits no linear relationship between dropsize and tear elimination coefficient is observed. The elimination coefficient is insensitive to the dropsize over the range of 1 to 10 µl and sometimes to 20 µl.

In general a dropsize smaller than 20 µl should be preferred to improve the therapeutic effect of ophthalmic drugs.

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INTRODUCTION

The bioavailability of drugs from topically applied solutions depends on the ocular contact time and the course of the amount of drug in the precorneal tear film. After instillation, drainage of the administered solution influences the activity of the therapeutic agent, since it removes the drug from the precorneal area, making it less available to exert a local effect or to be absorbed by the ocular tissues (1).

Chrai et al demonstrated in rabbits that the rate of drainage is related to the volume of fluid instilled. The rate increases with increasing volume. They suggested that to optimize the activity of ophthalmic drugs in humans, the instilled volume should be reduced to 5 or 10 µl dropsize, instead of 50 µl drops delivered by commercial dispensers (1).

The aim of the present study is to investigate the influence of the dropsize on the precorneal retention of a tracer in human eyes. A non-invasive method causing minimal disturbance was chosen. A fluorescent tracer is applied into the conjunctival sac and the fluorescence decay in the tear film is monitored with a slit lamp fluorophotometer (2).

MATERIALS

1. Tracer Solutions.

The tracer selected is sodium fluorescein, because of its low toxicity and high fluorescence efficiency. Fluorescein absorbs light in the blue region of the visible spectrum with a peak near 490 nm and fluorescences in the green region with a peak near 520 nm (3).

Fluorescein is not resorbed through the intact corneal and conjunctival epithelium. It follows the normal route of drainage of the lacrimal fluid (3,4).

The concentration of the tracer solution chosen is based on the dilution obtained by the resident lacrimal fluid. The output of the fluorescence signal is situated in the linear response range of the slit lamp fluorophotometer (2).

The aqueous tracer solutions are prepared by dissolution of sodium fluorescein (Fluka) in normal saline vehicle and afterwards aseptic filtration. The osmolality of each test solution is determined using a Knauer halb micro osmometer (Knauer, Eppelheim, FRG). The characteristics of the tracer solutions are listed in table 1.

2. Instrumentation.

To measure the decay of fluorescein in the precorneal tear film a fluorophotometer was developed by modifying a Haag Streit 360 slit lamp. A schematic diagram of the instrument is drawn in FIG. 1.

The light source is the standard incandescent light bulb, powered by a regulated voltage supply. In order to be able to work at day light a "lock - in amplifier" principle was used. To implement a coherent or synchronous detector system, the excitation light is modulated at 333 Hz by chopping the beam. A photodiode in the chopped beam is used to generated the required reference signal. The exciter filter (E) (Zeiss Erregerfilter 485) is mounted in front of the chopper-diode unit (CPU). The illumination arm and the microscope arm are locked at an angle of 45° from each other.

The photomultiplier (PM) is mounted directly onto an eye-piece of the slit lamp, while the left ocular of the binocular microscope system enables observation of the eye during the experiment. The blue light of the excitation beam from the slit lamp causes fluorescence of the tracer in the tear film. This fluorescence is detected by the photomultiplier through a barrierfilter (B) (Zeiss Sperrfilter 520). The photomultiplier current is converted by the convector unit (CU) into a voltage (SIG) and fed together with the reference signal (REF) into the lock-in amplifier (Brook-

TABLE 1. Characteristics of the Tracer Solutions.

Dropsize (µl)	Concentration Tracer (%)	Osmolality (mosm/kg)
1	0.5	355
5	0.1	305
10	0.1	305
20	0.05	294

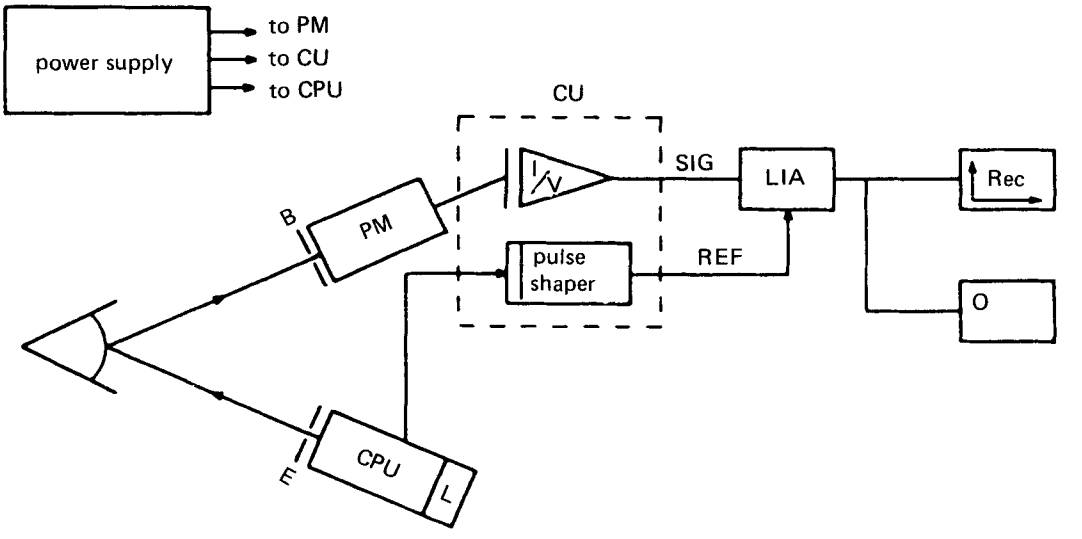

FIG. 1. Schematic diagram of the fluorophotometer.
Legend : L = lamp ; CPU = chopper-diode unit ;
E = exciterfilter ; B = barrierfilter ;
CU = convertor unit ; O = oscilloscope ;
LIA = lock-in amplifier ; SIC = signal ;
REF = reference signal ; REC = chart recorder.

deal type 401). The output is displayed on an oscilloscope (O) and a x/t recorder (REC) (Philips PM 8125).

The more detailed specifications of this instrument may be found in an earlier report (2).

METHOD

The trials were carried out in 4 male volunteers, without ocular disorders, aged 30-40 years. Informed consent was obtained from all participants, after the nature of the procedure was fully explained.

The subject is seated in front of the slit lamp and positioned on a chin and head rest. He fixates on a target to ensure stability of the eye. The size of the excitation beam illuminated on the eye is 2 x 8 mm (width by height). Recordings of the tear film fluorescence are made centrally near the limbus on the sclera at the lateral canthus. For the subjects comfort and also to avoid blue light hazards on the retina, measurements are not made at the center of the cornea (3). Prior to the experiments the autofluorescence of the conjunctiva and sclera is registered. This background fluorescence signal is substracted from all subsequent readings.

The instilled volumes were selected based on the work of Mishima et al. (5). The administration of a 20 µl drop results in a resident volume of 27 µl fluid, assuming a normal lacrimal fluid volume of 7 µl. Mishima reported that the human eye can only holds about 30 µl fluid without spillage and overflow on the check. Therefore tracer solutions ranging from 20 to 1 µl are administrated carefully to the subjects.

At zero time the required volume of tracer solution is applied into the conjunctival sac of the left eye with a Gibson pipette, avoiding contact to ocular tissue and eye lashes. Immediately after instillation the subjects are asked to close and roll their eyes during 5 seconds in order to mix the tracer with the resi-

dent fluid. Afterwards the monitoring of the tear film fluorescence starts. The volunteers are allow to blink freely as they feel necessary during the monitoring over a period of 4 minutes. The net fluorescence of the tear film as a function of time is analyzed and the percentage fluorescence decay is calculated.

The volunteers underwent six trials on succesive days or a few days interval.

RESULTS

1. Decay profile.

Typical plots of the change in the fluorescence of the tear film are drawn in FIG. 2 and 3.

Following topical application of the tracer solution, the amount of tracer and consequently the tear film fluorescence decline very rapidely. The decay profile has a monophasic (FIG. 1) or biphasic pattern (FIG. 2), depending on the volunteer.

In the majority of cases an initial rapid decay is followed by a subsequent slower elimination after a few minutes. Thus the eye is only exposed during a short period of time to a high amount of tracer.

The rapid fluorescence decay is due to two processes occuring simultaneously. Firstly the added volume in excess drains out, until it is reduced to the normal lacrimal volume. This drainage caused by blinking induces a rapid loss of the tracer from the precorneal area. Secondly the applied tracer solution is diluted by basal tear production, but especially by reflex tearing induced by the instillation of the eye drop.

The instillation of various volumes of a non-irritating tracer solution seems to cause a physiological and reflex reaction and also blinking.

2. Tear Elimination Coefficient.

According to Mishima and to Sørensen the initial phase of the decay profile is fairly well fitted to the exponential pattern

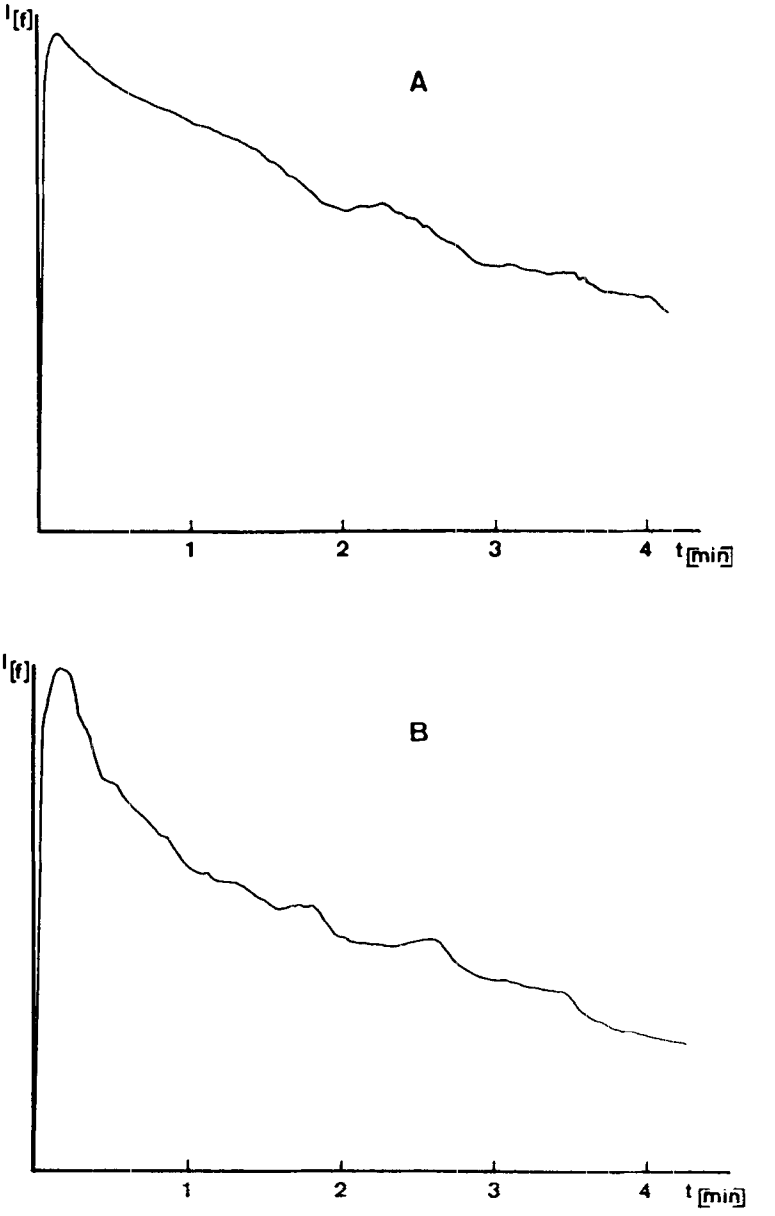

FIG. 2. Fluorescence decay profile (volunteer 1)
instilled volume : A = 1 µl ; B = 5 µl.

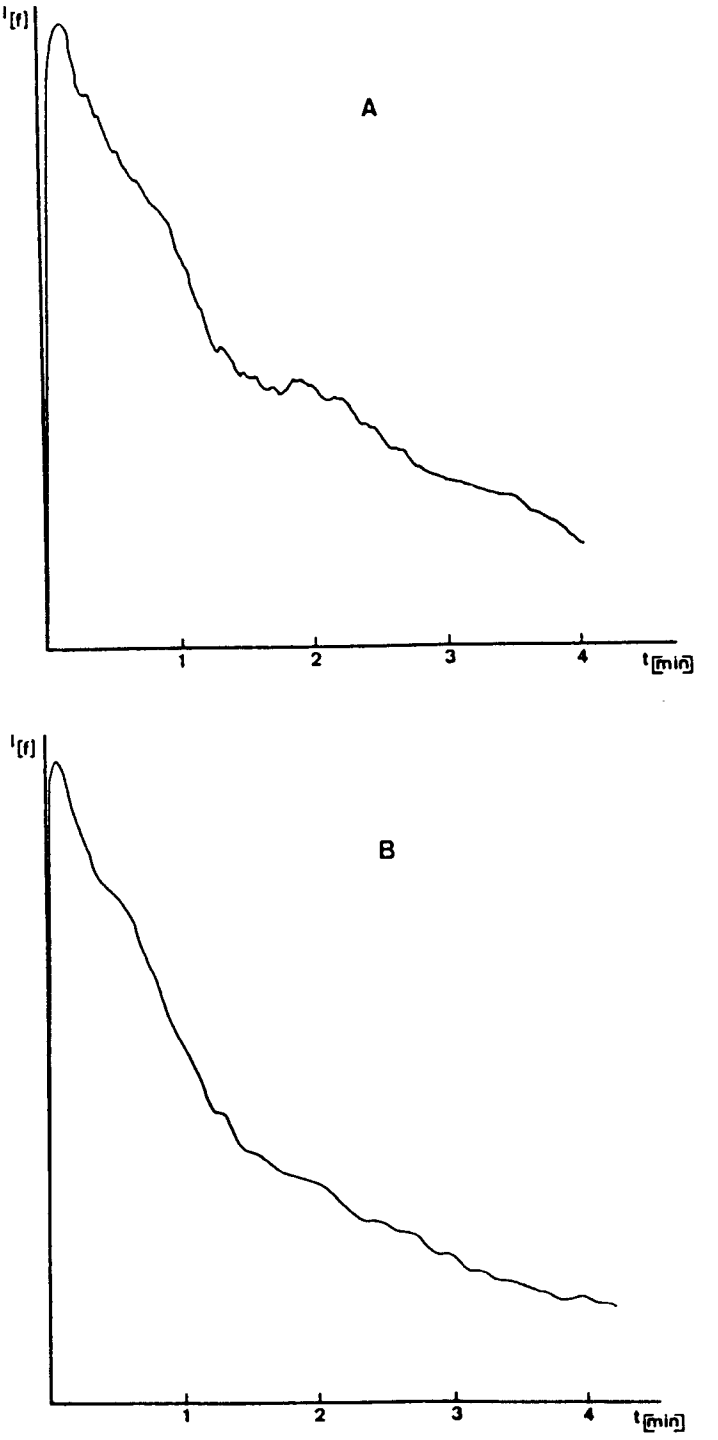

FIG. 3. Fluorescence decay profile (volunteer 2)
instilled volume : A = 1 µl ; B = 5 µl.

TABLE 2. Tear Elimination Coefficient k (sec^{-1}).

Dropsize	1 µl		5 µl	
	k	SD	k	SD
Volunteer				
1	0.0043	0.0018	0.0065	0.0013
2	0.0095	0.0030	0.0105	0.0035
3	0.0080	0.0034	0.0108	0.0019
4	0.0109	0.0044	0.0097	0.0022
Dropsize	10 µl		20 µl	
	k	SD	k	SD
Volunteer				
1	0.0059	0.0022	-	-
2	0.0137	0.0031	0.0135	0.0040
3	0.0139	0.0022	0.0120	0.0030
4	0.0079	0.0022	0.0137	0.0041

of elimination (5,6). The data are subjected to linear regression analysis by the methode of least squares. The first order rate constant for the disappearance of the tracer from the precorneal area of the eye, called tear elimination coefficient k, is calculated for each experiment. The mean k value of six trials and the standard deviation are summarized in table 2.

Volunteer 1 could not hold a drop of 20 µl, without spillage and overflow of tears. Therefore those data were withdrawn.

Large intra and inter variations are observed among the volunteers. Some SD values appear to be quite large, demonstrating the variability of the decay for succesive measurements on the same subject.

TABLE 3. Significance Test - one way analysis of variance - of the Tear Elimination Coefficient.

Dropsize comparisons	Volunteers			
	1	2	3	4
20 µl vs 10 µl	-	NS	NS	$P < 0.01$
5 µl	-	$P < 0.01$	NS	NS
1 µl	-	$P < 0.01$	$P < 0.05$	NS
10 µl vs 5 µl	$P < 0.05$	$P < 0.01$	NS	NS
1 µl	NS	$P < 0.01$	$P < 0.01$	NS
5 µl vs 1 µl	NS	NS	NS	NS

The results of the statistical analysis of the data of each volunteer separately are given in table 3.

Thus the general trend seems to be a faster elimination when drops of 20 µl or in some cases 10 µl are instilled compared to small drops of 5 and 1 µl.

DISCUSSION

From the present study no linear relationship between dropsize and tear elimination of the tracer is observed. Chrai et al.,however, demonstrated in rabbits a linear relationship. The rate of drainage increases with increasing instilled volume over a range of 5 to 50 µl (1). But Lee noted in rabbit eyes, that the rate constant of elimination of inulin liposomal preparations from the lacrimal fluid is insensitive to the volume of the instilled preparations over the range of 10 to 50 µl (7). Also Sørensen showed in human volunteers that the shape of the elimination curves of an ophthalmic solution containing a radioactive tracer is not influenced by a dropsize of 5 or 15 µl (6).

The difference between the work of Chrai and the present study may be due to the much lower blinking frequency of rabbits compared to man, resp. 4 to 6 times/h and 600 to 1200 times/h (7).

Several authors pointed out the role of blinking for effective drainage. Therefore the influence of blinking on the fluorescence decay is investigated. Appropriate settings of the lock-in amplifier and the recorder are use for this study. Also a 0.5 % fluorescein solution in normal saline is instilled, using the same procedure. From the recordings taken 10 minutes after instillation during the stable basal elimination phase, the time between blinks can be deduced (2).

The time interval between blinks is not constant, even for the same subject, and varies from 7 to 35 seconds. The wide fluctuations are in agreement with other studies (9-12). Volunteers exhibiting a rapid tracer elimination have mostly a short blink interval (7 to 10 sec). These results illustrate the importance of blinking on the elimination of the tracer. The sensitivity and the individual reflex reaction to the instillation of eye drops result in large inter subject variations.

CONCLUSIONS

After instillation in a conventional manner, an ophthalmic solution drains away very rapidly from the eye, causing an important loss of drug. The instillation of eye drops provoke a reflex reaction, resulting in blinking and reflex tearing.

The present study indicates that the elimination coefficient is insensitive to the dropsize over a range of 1 to 10 µl, sometimes to 20 µl. Therefore a dropsize smaller than 20 µl should be preferred because of a slower elimination and consequently a longer contact time and a higher availability of the drug in the precorneal area of human eyes.

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