Ocular contact time of a carbomer gel (GelTears) in humans

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Abstract

***Background/aims*—Carbomers are widely used in products for the treatment of dry eye; however, the polymer gel thins on addition of probes (for example, fluorescein salt) confounding the comparison of products by objective clinical tests such as spectrophotofluorimetry or scintigraphy. A novel method of radiolabelling carbomer gels, with minimum change to their rheology, has permitted the non-invasive evaluation of precorneal residence of the gel in volunteers using gamma scintigraphy. The technique was used to evaluate the precorneal clearance of the liquid phase and of a suspended particulate in GelTears.**

***Methods*—Low sodium technetium-99m labelled diethylenetriaminepentacetic acid (^{99m}Tc-DTPA) was used to label carbomer 940 gel, either adsorbed onto sterile charcoal to model an entrapped drug, or added directly to the gel to a final activity of 1 MBq per 25 µl dose. The clearance of the labelled gels was then compared with ^{99m}Tc-DTPA labelled saline in 12 volunteers.**

***Results*—The addition of the low sodium radiopharmaceutical produced insignificant rheological changes in the gel compared with conventional ^{99m}Tc-DTPA labelling. The residence times on the eye of the gel formulations were significantly greater than that of the saline control. At 8 minutes postdosing, the label levels retained (mean (SD)) on the ocular surface were: saline, 7% (7%); ^{99m}Tc-DTPA gel, 42% (27%); and ^{99m}Tc-carbon gel, 42% (20%) of administered dose. There was no difference observed in the precorneal distribution between ^{99m}Tc-DTPA solution and particulate markers.**

***Conclusions*—These data demonstrate that carbomer based gels significantly extend contact of solutes or suspended solids with the corneal surface. The method of labelling does not significantly change the initial viscosity and is superior to previous methods which have used sodium salts (for example, sodium fluorescein) and therefore underestimate contact time.**

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Studies in humans have shown that polymer excipients are effective in the treatment of dry eye syndrome. The role of the polymer is thought to relate to the ability to moisten the epithelial surface and to provide structure for the aqueous phase of the tear film in a manner analogous to natural mucins. High molecular weight polymers, such as hyaluronic acid, have been shown to slow the turnover of the preocular film.[1 2] Application of hyaluronate at 0.3% w/v concentrations caused an increase in tear film thickness within a few seconds of application which was maintained above baseline for over 30 minutes. Until recently, this material has been regarded as being too expensive for commercial development as an artificial tear product, especially as cheaper alternatives exist.

Marquardt and Christ compared the residence of a polyacrylic acid (carbomer) based gel with a polyvinyl alcohol based preparation, measuring the fluorescence of the precorneal tear film in healthy volunteers after instillation of the polymer containing sodium fluorescein as a marker. The authors measured tear break up times and conducted Schirmer tests in a small group of patients. Data were obtained which suggested a positive pharmacodynamic effect up to 6 hours post-dose.[3] However, in the presence of cations, especially sodium or calcium, the viscosity of carbomer gels changes markedly. Unlu and coworkers reported significant rheological changes on incorporation of 0.05% w/v sodium fluorescein (0.05%) and 0.01% w/v disodium edetate.[4] This suggests that in the previous studies the rheology would have been significantly altered by addition of the probe. Our pilot studies showed that addition of technetium-99m (^{99m}Tc)-sodium pertechnetate in isotonic saline at concentrations of 4–10% v/v caused visible viscosity changes in the vehicle.

In the present study, gamma scintigraphy has been utilised to assess the precorneal residence of a radiolabelled carbomer formulation (GelTears, Chauvin Pharmaceuticals, Romford) in healthy male and female volunteers. In order to radiolabel the formulation, it was necessary to develop a novel method of concentrating the radiolabel and adding it to the formulation at the lowest concentration of sodium possible. The method developed appears to be suitable for the general purpose labelling of carbomer gels with ^{99m}Tc labelled DTPA, and was also used to label carbon particles which were incorporated into the formulation. This enabled a comparison between the ^{99m}Tc-DTPA label in saline, the gel labelled in the aqueous phase, and a gel in which carbon particles mimicked macromolecular species in the formulation.

Methods

PREPARATION OF LABELLED FORMULATIONS

Technetium-99m was prepared in the form of ^{99m}Tc-sodium pertechnetate by elution in sterile saline from a Mo-99/Tc-99m generator (CIS (UK) Ltd). An aliquot of the eluate (0.25 ml, 3 GBq) was extracted into an equal volume of n-butanone. The organic supernatant was transferred to a clean vial and dried under a stream of warm dry air in a laminar flow cabinet. The residue was redissolved in 100 µl DTPA solution (Technescan, Mallinchrodt Medical) reconstituted with sterile water. This modified ^{99m}Tc-DTPA adduct was then used for radiolabelling the carbomer 940 gel (GelTears).

Saline/^{99m}Tc-DTPA or GelTears/^{99m}Tc-DTPA

Radiolabelled saline solution or the GelTears formulation was obtained by the addition of 10 µl of low sodium ^{99m}Tc-DTPA to 2 g of sterile saline solution or sterile GelTears (specific activity of 1 MBq per 25 µl dose at time of administration).

GelTears/charcoal/^{99m}Tc-DTPA

Sterile carbon 50 mg (mean particle size (mm)) was mixed thoroughly using a sterile spatula with 10 µl of low sodium ^{99m}Tc-DTPA solution and dried. The radiolabelled carbon was then added to 2 g of the GelTears formulation and mixed to obtain content uniformity (specific activity of 1 MBq per 25 µl dose at time of administration). All procedures were carried out in a laminar flow cabinet under good laboratory practice conditions.

RHEOLOGY OF LABELLED GELTEARS

A Ferranti–Shirley cone and plate viscometer with attached X-Y plotter was used to examine the rheological properties of radiolabelled GelTears samples. A 20 minute sweep time with speed of 100 rpm and a large cone of radius of 3.75 cm was used in the present study.

GAMMA SCINTIGRAPHY

Subjects attended a prestudy visit in which they completed a survey of their general health and underwent a standard physical investigation.

On acceptance into the trial, 12 subjects (four females and eight males) attended the research clinic 30 minutes before administration and received the formulations into one eye only, according to a predetermined randomised allocation sequence. Subjects were dosed while seated and the material applied using a positive displacement pipette. The head was tilted and the eyelids gently held apart to avoid interference from the lashes. With the head erect and positioned in a chin rest, images were acquired on a Maxicamera II gamma camera (General Electric, USA) using the 140 keV photopeak of technetium and a pinhole collimator. A dynamic sequence of images was taken according to the following sampling schedule: 96 frames at 5 seconds' dynamic acquisition followed by static acquisitions at 15, 25, and 35 minutes postdose.

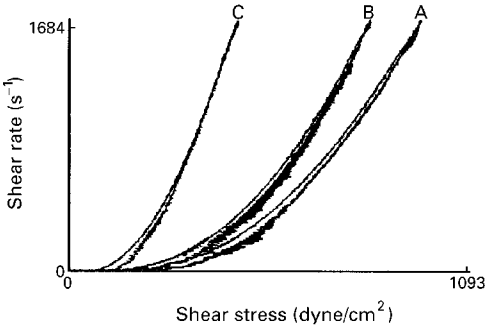

Figure 1 The effect of sodium ion on flow curves of GelTears. (A) GelTears, η'=1.93 cps; (B) GelTears with low sodium ^{99m}Tc-DTPA, η'=1.77 cps; and (C) GelTears with isotonic sodium ^{99m}Tc-DTPA, η'=0.72 cps, where η' is apparent viscosity at shear rate 1684 s^{-1}. Note the addition of isotonic saline to the carbomer causes a marked reduction in apparent viscosity.

On successive visits 3 days apart, volunteers received alternate formulations. All data were backed up and stored on optical disk for further analysis.

DATA ANALYSIS

Images were recalled from the computer and analysed using the NUD image analysis program. The first 25 frames were summed to provide an anatomical image of the whole eye in order to provide a template to construct regions of interest. The lower threshold on the visible display was set at 20% to exclude the visible background activity; however, this did not exclude background counts from being included in the image quantification. In all cases this image provided sufficient detail to determine the position of the cornea/sclera, inner canthus, and lacrimal duct. Separate regions of interest (ROIs) were created for each of the three visits for each subject to allow for variations in positioning on each occasion. The individual count rates, number of image cells, and frame times were tabulated and recorded for each frame in the study. The data were then analysed on a validated spreadsheet for calculation of the precorneal residence in the three ROIs. The data were then expressed as a percentage of the administered dose to obtain the percentage retention with time and averaged across treatment groups to obtain mean clearance curves.

For measurements of formulation residence in each subject, the area under the curve (AUC) was calculated by the trapezoidal rule from the first to the last dynamic ($AUC_{0\text{-}480s}$) and the first dynamic until the last static view ($AUC_{0\text{-}2100s}$).

Results

RHEOLOGY OF THE LABELLED GELS

Rheograms of the labelled GelTears formulation were derived at scale 4 (as shown in Fig 1) and apparent viscosities were calculated at loop apices (1684 s^{-1}).

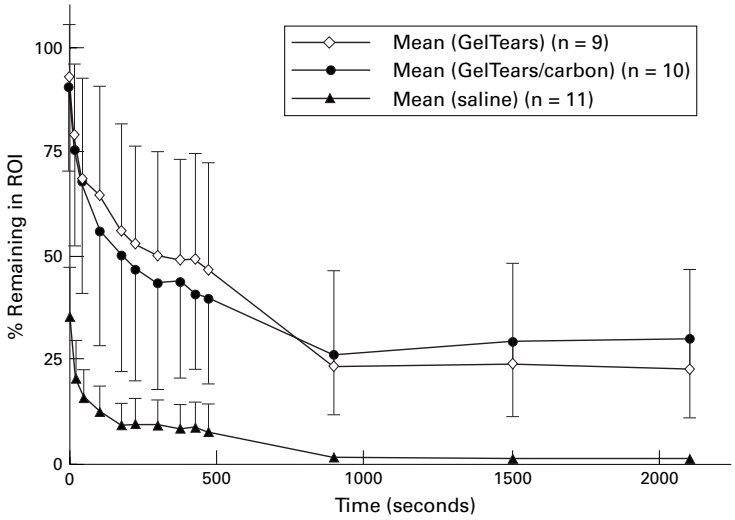

Figure 2 Mean corneal clearance of GelTears or saline formulations labelled with ^{99m}Tc-DTPA in the solution or particulate phase (mean (SD)).

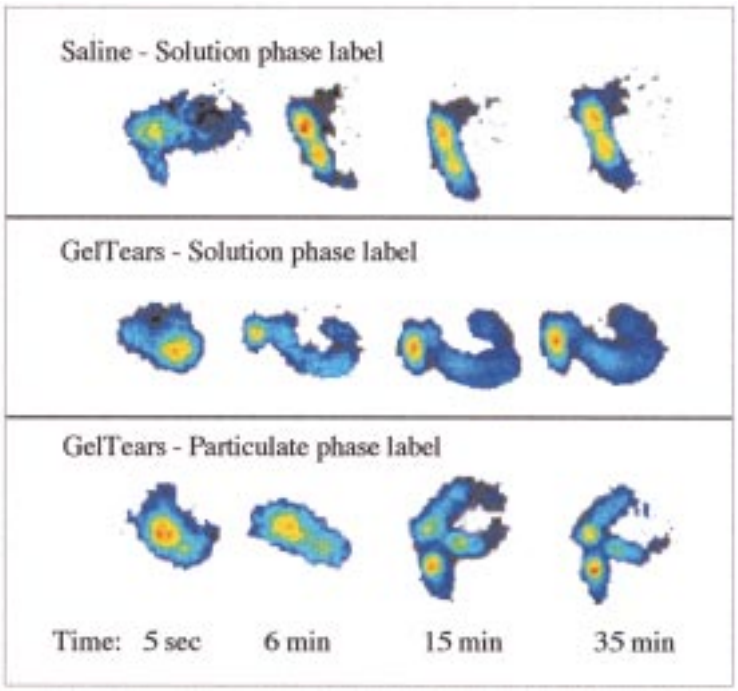

Figure 3 Typical scintigraphic imagines of GelTears and saline control obtained during the study.

Table 1 Mean AUC values (SD) of gel formulations and saline in humans

Preparation	*$AUC_{0\text{-}480s}$ (% min)*	*$AUC_{0\text{-}2100s}$ (% min)*
GelTears	406 (215)	983 (681)
GelTears with carbon	402 (192)	1115 (559)
Saline	87 (41)	Non-detectable

SCINTIGRAPHIC RESULTS

Of the 12 subjects entered into the study, 11 completed the three treatments. No untoward effects were reported by the subjects, who described the formulations as "comfortable". At the end of the study, the eyes were healthy with no redness or signs of irritation. All 11 were analysable in the saline treatment group but overspill prevented accurate analysis in one subject in the ^{99m}Tc-DTPA carbon labelled formulation and two in the ^{99m}Tc-DTPA solute labelled formulation groups. Eight subjects provided data analysable for all treatments following dynamic acquisition. Between 15 and 35 minutes, seven of the ^{99m}Tc-DTPA solute and nine of the ^{99m}Tc-DTPA carbon labelled formulation were suitable for analysis. Mean data are presented in graphical form in Figure 2 and AUC data are listed in Table 1. Representative scintiscans are shown in Figure 3 for the three treatment groups.

Discussion

A comparison of the apparent viscosities of GelTears labelled with ^{99m}Tc-DTPA in isotonic sodium chloride solution, low sodium ^{99m}Tc-DTPA, and the unlabelled product showed that the rheological behaviour of the labelled GelTears was altered on addition of the label. However, the rheogram was closer to the original product in the case of the low sodium ^{99m}Tc-DTPA. Unlu and coworkers[4] found that the addition of 0.05% sodium fluorescein or 0.10% w/v disodium edetate caused a significant change in the flow properties of the polymer. These fluorescein concentrations are also similar to those used by Marquardt and Christ,[3] who labelled the carbomer preparation by addition of 0.14 ml 5% sodium fluorescein to 10 g preparation. Here, the use of the n-butanone extraction step significantly reduced the sodium concentration added to the gel, thereby maintaining the gel strength. We have found this method is suitable for the labelling of all carbomer based ophthalmic formulations studied to date.

When anionic polymers interact with mucin (which is also anionic), the maximum interactive adhesive force occurs at an acid pH, suggesting that the mucoadhesive in its protonated form is responsible for the mucoadhesion. The observed precorneal residence of the carbomer formulation is due to this type of interaction.[5] The swollen polymer entangles with mucin on the eye, stabilising a thick hydrogel structure. The label, ^{99m}Tc-DTPA, remains in the aqueous phase and is eliminated as the polymer erodes, dissolves, and collapses. The retention of the labelled gel, as measured from the solute or particulate phase marker, shows more than 40% of the label was associated with the cornea, 10 minutes after dosing. The lacrimal ROI shows the corresponding low rate of nasolacrimal drainage with significant differences between the gel and saline formulations. In some subjects, a very thick layer was maintained, with a sudden loss of material at 10–15 minutes. This material overspilled onto the lash and cheek and in these subjects precise definition of the corneal ROI was not possible. Data from these subjects were therefore not analysed. The data obtained in the study suggest that the formulation remains associated with the cornea for significant periods of time. At 35 minutes, approximately 20% of the activity is associated with the cornea, suggesting stabilisation of tear film for a considerable time.

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