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Dry powder dosing in liquid vehicles: ocular tolerance and scintigraphic evaluation of a perfluorocarbon suspension

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Abstract

The ocular tolerance and precorneal disposition of ^{99m}Tc-labelled sterile carbon-perfluorodecalin (PFD) and carbon-aqueous suspensions were examined in a cohort of healthy volunteers. Formulations were prepared in PFD or saline using charcoal particles, radiolabelled with [^{99m}Tc]diethylenetriaminepentaacetic acid (DTPA) under GMP conditions. Colloidal silicon dioxide was used as a suspending agent. Ocular tolerance was examined following the instillation of each formulation to the eyes of 12 volunteers. The precorneal distribution of both formulations in man was monitored using gamma scintigraphy. Dynamic and static data acquisitions were taken over a period of 150 min after dosing. Carbon particulates suspended in PFD did not show any irritation to the eye. Administration of PFD formulation in man produced a significant increase in ocular retention over a saline formulation (mean residence time (MRT) = 157 ± 42 and 0.29 ± 0.08 min, respectively, $P = 0.0001$). Distribution of the carbon in man followed the same pattern as in a previous reported study in animals. The carbon deposited uniformly along the lid margin in the case of the PFD vehicle, whereas it agglomerated following dosing in the saline vehicle and was ejected from the eye. The novel non-aqueous vehicle system is able to significantly improve the ocular retention of charcoal particles in man and provides a unique distribution of the particles in the eye, which suggests a potential for the PFD system for the treatment of periocular diseases.

Keywords: Perfluorodecalin; Non-aqueous vehicle; Tolerance; Scintigraphy; Precorneal distribution; Volunteers

Abbreviations: AUC, the area under the activity remaining in ROI-time curve; Cab-O-Sil®, colloidal silicon dioxide; DTPA, diethylenetriaminepentaacetic acid; MRT, mean residence time; PFD, perfluorodecalin; ROI, region of interest; Tc, technetium.

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1. Introduction

Poorly-soluble drugs for ophthalmic administration are frequently formulated as micronised suspensions. Such preparations offer the advantage of prolonged corneal residence, associated with an increased bioavailability. Larger particles theoretically provide a prolongation of effect due to the increased size of the reservoir; however, an increase in particle size is associated with irritation giving rise to an increased rate of removal, assisted by agglomeration of particles and ejection. Schoenwald and Stewart (1980) demonstrated that for 0.1% [^{3}H]dexamethasone, administered as suspensions with mean particle sizes of 5.75, 11.5 and 22 μm, dissolution at the greater particle size proceeded so slowly that the particulates were ejected from the eye before dissolution was complete.

A suspension placed into the eye will first encounter mucin in the tear film. Subsequent coating of particles with the glycoprotein will normally agglomerate the suspension into a mass. Conversely if a dry powder such as kohl is applied to the corneal/conjunctival membrane, it will adhere to the lid margins avoiding the tendency to clump. Kohl is cosmetic powder used originally in Muslin and Asian countries to darken the area around the eyes. It is usually powdered antimony sulphide. This provides a pleasant cosmetic effect, exploited by many cultures to draw attention to the eye. A previous investigation conducted in rabbits (Zhu et al., 1999) revealed that the application of perfluorocarbon-based suspension into the eye resulted in an even distribution of material along the conjunctival margin. Lagomorphs possess an accessory structure, the nictitating membrane, which renders the anatomy and physiology of the eye very different to man. It was therefore important to establish the behaviour of the novel perfluorocarbon preparation in a clinical investigation. Following a tolerance study to assess irritant potential, lacrimal scintigraphy was carried out in 12 normal volunteers. The model particulate, activated, micronised charcoal, provided a visible and measurable marker on the ocular surface. The carbon was radiolabelled with technetium-99m labelled diethylenetriaminepentaacetic acid ([^{99m}Tc]DTPA), formulated in saline and perfluorodecalin (PFD) vehicles and examined in a randomised cross-over design.

2. Materials

Perfluorocarbon, colloidal silicon dioxide and micronised carbon were used as supplied by Allergan®. [^{99m}Tc]sodium pertechnetate was prepared by elution of an Elumatic III technetium-99m generator (CIS International) using a vacuum vial to yield a solution containing approximately 12 GBq activity in 2 ml. The eluate was added to a kit for the preparation of ^{99m}Tc-labelled diethylenetriamine-pentaacetic acid ([^{99m}Tc]DTPA) (Pentetate®, Amersham Radiopharmaceuticals, UK).

Manufacture of the formulations was carried out in an aseptic suite. Radiolabelled carbon was prepared by soaking sterile activated charcoal in saline containing [^{99m}Tc]DTPA. The carbon was then freeze-dried overnight using a freeze dryer (Edwards MiniModulo) which had been instrumented by fitting a thermocouple on the condenser and by piggybacking the Pirani vacuum gauge onto existing instrumentation. This allowed pressure and temperature recording to a data acquisition station fitted to a desktop computer. Colloidal silicon dioxide was added to small volumes of PFD and the carbon added under aseptic conditions. The mixtures were then sonicated for 10 min to produce a suspension.

3. Methods

3.1. Comfort and tolerance study

Approval from the University of Nottingham Medical School Ethical Committee and ARSAC certification was obtained to carry out this study in normal volunteers. Written and verbal details of the study were given to all subjects (seven females and five males, with an age range of 20–30 years) and informed consent was obtained prior to the trial.

On the study days subjects were asked to remain in the study room, which had controlled lighting (no windows) and controlled temperature, in order to minimise variation due to external factors during the experiment. None of the subjects wore eye cosmetics on the study day. A single 8-μl drop of either the saline/carbon or PFD/carbon formulation was administered to the cornea of the left eye using a positive displacement pipette fitted with a sterilised disposable tip. Subjects were then asked to remain in the room and responses were recorded periodically using a questionnaire. The ocular responses with special regard to reflex lacrimation, red eye, or other ophthalmic symptoms following dosing, were determined by an ophthalmologist. In three subjects with suitably light blue eyes, slit-lamp photographs of the treated eye were taken prior to the start of the study and at 1 min and 30 min post dosing to assess the distribution and periocular vascular response of the eye to the formulation. The evaluation included, but was not limited to, the conjunctiva, cornea, anterior chamber and iris. The duration of the study was 2 h; however, there was the option to extend the study period in the event of an adverse reaction. After a week washout period, subjects were dosed with the alternate formulation according to the randomisation code, and the comfort questionnaires filled out as before.

3.2. Gamma scintigraphic study in man

The same 12 normal volunteers subsequently participated in a scintigraphic investigation to study the precorneal residence of the ^{99m}Tc-labelled formulations in the eye. A randomised allocation, cross-over design was used. On both study days, each subject sat in front of the gamma camera fitted with a 3-mm pinhole collimator. The head was supported on an ophthalmic table. The left eye was positioned in line with the pinhole at a distance of 5 cm from the aperture. A single drop (8 μl) of either saline/carbon or PFD/carbon formulation containing the radiopharmaceutical (1 MBq) was placed in the left eye using a positive displacement pipette fitted with a sterilised tip. The formulations were administered to one eye only with the other being a control. The precorneal distribution of the drop was assessed using a gamma camera (IGE Maxicamera, 400T) with gamma radiation energy between 343 and 443 keV. A protocol consisting of a dynamic sequence of 60×10 frames was employed followed by a series of static images taken at various time intervals up to 4 h post instillation or until activity decreased to the background level. No drug or anaesthetic was administered during the experiment. Examinations of static images for lash and skin contamination were made, and the carbon from exterior of the eye was removed using a moistened cotton bud as necessary.

The data were stored on computer for later analysis. There were five regions of interest (ROI): cornea, inner canthus, lacrimal duct, whole eye and the background. The counts for each region in each frame of study were determined and corrected for movement, background counts and isotope decay. Graphs of percentage remaining as a function of time were plotted.

4. Results

4.1. Comfort and tolerance study

Of the 12 volunteers participating in this trial, three noted discomfort though only one subject felt sufficient sensation to cause him to register the effect on his questionnaire. One subject complained of slight grittiness on initial dosing with both the saline and the PFD; 20 min later he experienced a slight dryness in the dosed eye. The second subject commented on the initial feeling of grittiness when dosed with the saline and the third one mentioned a tickly feeling 10 min into the study. This subject had also been dosed with the saline formulation.

In our routine surveillance of the subjects, no reflex tearing or redness was observed following treatment with either formulation. However, charcoal was seen to be ejected into the inner canthus or onto the lids in some volunteers who were dosed with the saline formulation.

Slit lamp photographs, taken of three of the subjects, confirmed absence of irritation, redness

or reflex tearing on dosing with either of the formulations. As can be seen in Fig. 1, carbon deposited uniformly along the lid margin in the case of PFD, whereas it agglomerated within 1 min following dosing of the saline vehicle and was often thrown out from the eye.

4.2. Gamma scintigraphic study in man

The mean ocular residence profiles of both formulations in man generated from dynamic and static studies are shown in Figs. 2 and 3. At 10 min following dosing with carbon/PFD system, $60.0 \pm 24.1\%$ (mean $\pm$ S.D.) of the radioactivity was found to remain on the corneal surface, whereas only $14.0 \pm 10.6\%$ activity of carbon/saline suspension was detected in the corneal ROI at the same time point. At 2 h post-dosing, there was still more radioactivity remaining on the corneal surface with the PFD formulation ($36 \pm 18.5\%$) than with the saline ($8 \pm 8.5\%$) formulation, indicating that the PFD had a significant sustained periocular retention of the carbon particles. The saline appeared to drain immediately away from the cornea following dosing and had the effect of drawing the charcoal to the caruncle. Approximately 30% radioactivity was found in the lacrimal duct 10 min after instillation of saline suspension. The PFD formulation appeared to markedly reduce the drainage to lacrimal duct since almost no activity ($\sim 3\%$) was found in the duct at the same time.

Typical scintigraphic images of both formulations are presented in Figs. 4 and 5. These illustrate the retention of the carbon on the periocular surface following dosing in the PFD formulation;

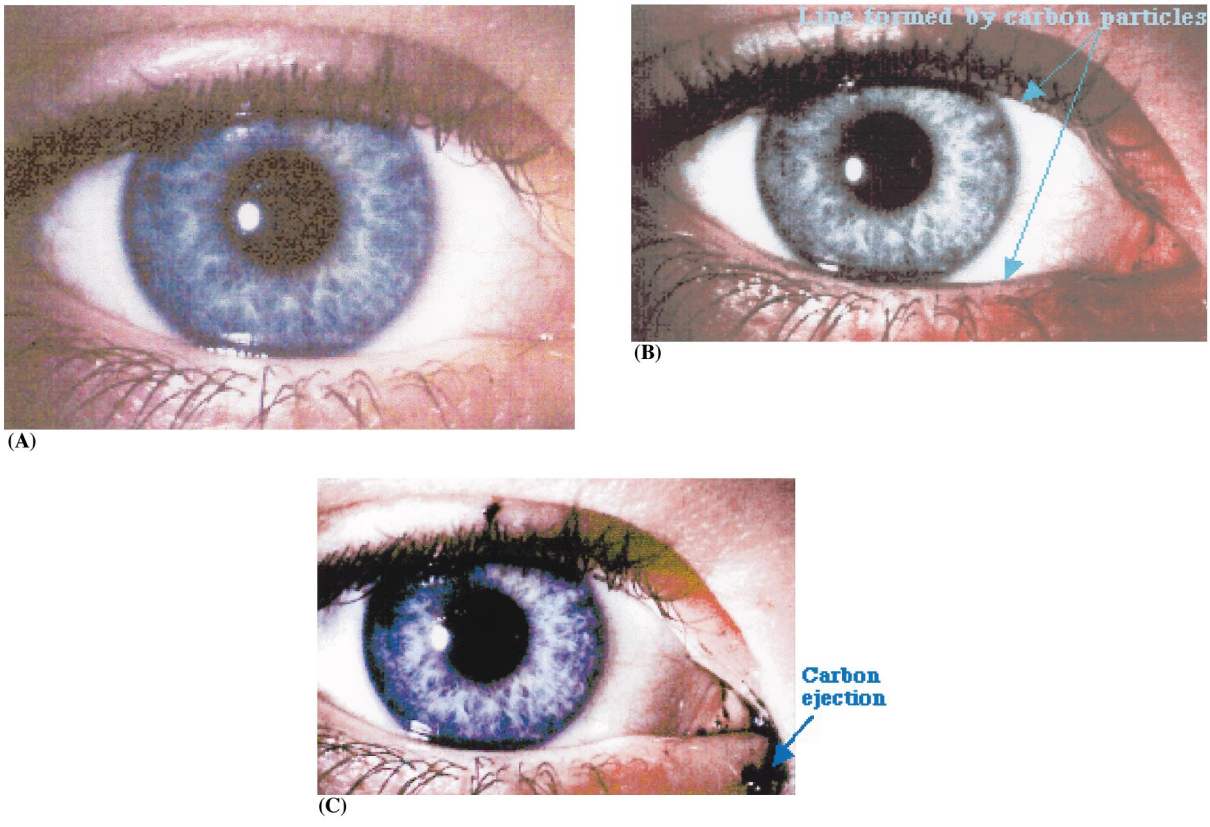

Fig. 1. (A) Normal eye at predose. (B) Photo of eye showing uniform distribution of PFD suspension at 1 min postdose. (C) Photo of eye showing carbon ejected over lower lid at 1 min after application of saline suspension.

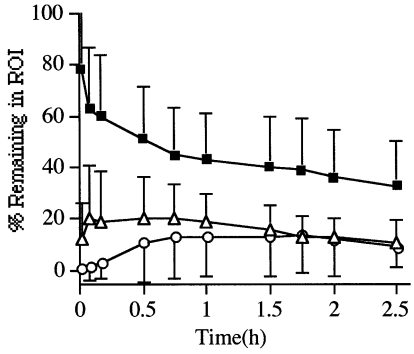

Fig. 2. The mean residence of PFD suspension in human eye (data generated from dynamic and static imaging, mean ± S.D., $n = 12$). —■— , cornea; —△— , inner canthus; —○— , lacrimal duct.

little drainage was observed in the nasolacrimal duct for up to 150 min post-dosing. However, almost all carbon particles were removed from the ocular surface at 10 min after dosing with saline formulation. Agglomeration and ejection was clearly noted, necessitating removal of exteriorised carbon with a moistened cotton wool bud.

The clearance of formulations from the cornea surface decreased bi-exponentially in most cases. An initial rapid phase of decline occurred in a period of 30 s after instillation, followed by a much slower elimination phase. This biphasic phenomenon could be partly explained by reflex tearing. The volume of reflex tears is reported to

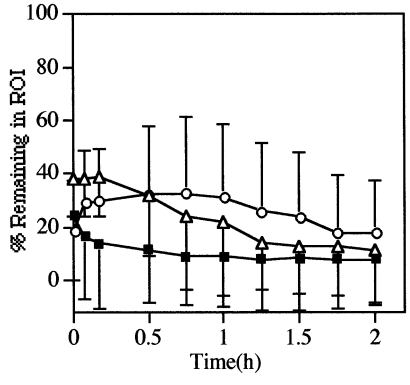

Fig. 3. The mean residence of saline suspension in human eye (data generated from dynamic and static imaging, mean ± S.D., $n = 12$). —■— , cornea; —△— , inner canthus; —○— , lacrimal duct.

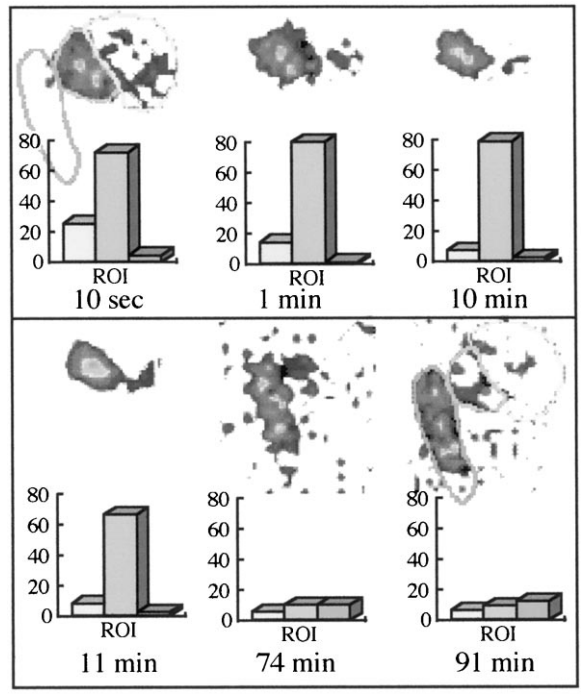

Fig. 4. The distribution of PFD suspension in the eye. Yellow, cornea; pale orange, inner canthus; dark orange, lacrimal duct.

be influenced by the irritating power of the instilled solution, and varies from 3 μl/min to 300–400 μl/min (Farris et al., 1981; Van-Ooteghem, 1987)

The mean residence time of both formulations ($MRT = AUMC_{0-\infty}/AUC_{0-\infty}$) was computed with a bi-exponential curve fit. These data gave satisfactory convergence and the calculated mean MRT (carbon/PFD) was 157 ± 42 compared to a mean MRT (carbon/saline) of 0.29 ± 0.08 min (mean ± S.E., $P = 0.005$).

The values of AUC of each subject for each formulation were calculated using the trapezoidal rule. The mean AUC was found to be 4.5 times greater for the charcoal/PFD system than for the saline formulation (5573 ± 658 and $147 \pm 30\%$ min; mean ± S.E., respectively). Analysis of variance of the $AUC_{0-120min}$ indicated that administration of the PFD in man produced a significant increase in residence compared to the saline formulation ($P = 0.0001$).

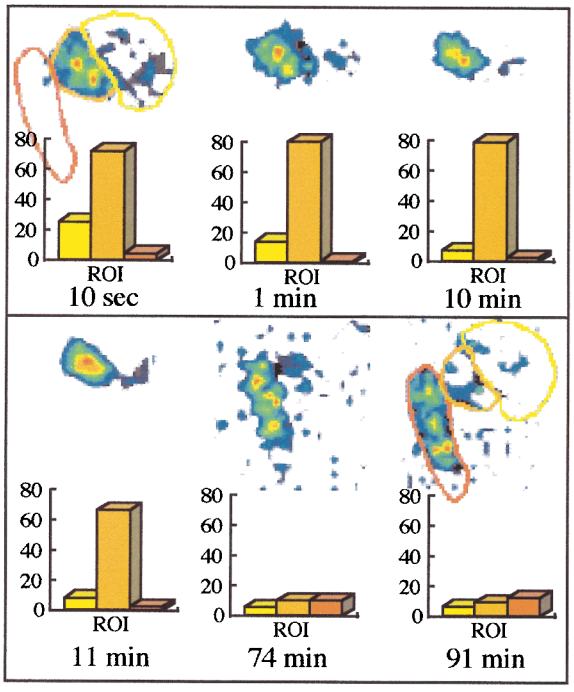

Fig. 4

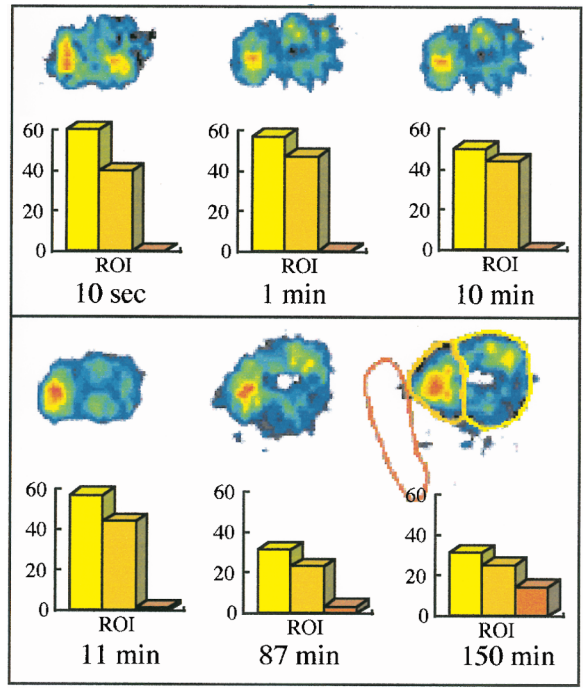

Fig. 5

Fig. 4. The distribution of PFD suspension in the eye. Yellow, cornea; pale orange, inner canthus; dark orange, lacrimal duct.
Fig. 5. The distribution of saline suspension in the eye. Yellow, cornea; pale orange, inner canthus; dark orange, lacrimal duct.

silicon dioxide surrounding the carbon particles acted to adhere the particulate to the surface. This approach should potentiate bioadhesion since hydration of a particulate close to the epithelial surface causes a strong association with surface bound mucins. Because of the time taken to hydrate the silica, the particulate distribution had reached the lid margins before full hydration had occurred. In contrast, in an aqueous formulation, the colloidal silica is fully hydrated and immediately aggregates in the presence of tears.

The potential of the PFD system in the treatment of diseases of the peripheral tissues such as blepharitis is immediately apparent. Two grey lines formed by charcoal following dosing with perfluorodecalin are seen to follow the lash lines, close to the apertures of the accessory Meibomian glands. In dry eye or keratoconjunctivitis sicca, an aggressive immunological response greatly reduces the amount of functioning tissue, leading to the failure to secrete oils and mucin needed for lubrication of the eye surface and the presence of dry spots where the mucin denatures. Concern has been expressed regarding the use of immunosuppressive agents such as cyclosporin in the treatment of this disease, since the agent is difficult to target to the eye margin and also it can not be formulated in aqueous vehicles due to the high lipophilicity.

The use of perfluorodecalin-based formulations also allows delivery of a small volume of eyedrops. A drop of 5–8 μl can easily be delivered in a PFD formulation using a standard dropper tip, due to its high density and low surface tension. Such a small instilled volume has shown a significantly reduced lacrimal drainage both in rabbits and man, at least for aqueous formulations. The nasal cavity is believed to be the main absorption site for most ophthalmic drugs. The reduced lacrimal drainage should result in decreased systemic absorption and therefore reduced systemic side-effects (Lutosky and Maurice, 1986; Whitson et al., 1993).

This should be significant for ophthalmic immunosuppressive agents, steroids and antiglaucoma agents which otherwise might cause unwanted systemic side-effects. Finally, patients on chronic treatment may develop allergic reactions to preservatives in ophthalmic formulations, which at high concentrations cause lysis of the cell membranes and opening of tight junctions. Non-aqueous suspensions based on PFD avoid the necessity of preservatives in the formulation, which may provide a significant advantage.

In conclusion, micronised particles suspended in the novel non-aqueous platform show strong adherence to the tear lash bases and conjunctival margins following dosing to the cornea. The distribution along the marginal strips suggests that particulate delivery can be targeted to accessory glands when dosed in this system. We believe that this unusual behaviour may be exploited to extend the boundaries of ophthalmic drug delivery.

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