Current Therapeutic Research 98 (2023) 100705

Contents lists available at ScienceDirect

Current Therapeutic Research

journal homepage: www.elsevier.com/locate/curtheres

Oxygen-Carrying Capacity of Perfluorohexyloctane, a Novel Eye Drop for Dry Eye Disease

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ARTICLE INFO

Article history:
Received 3 May 2023
Accepted 5 May 2023

Key words:
Cornea
Dry eye
Eye drop
NMR
Oxygen
Perfluorhexyloctane

ABSTRACT

Objective: One-hundred percent perfluorohexyloctane (PFHO) is a water-free, preservative-free eye drop approved by the Food and Drug Administration in the United States for the treatment of dry eye disease. PFHO has shown relief of dry eye signs and symptoms in clinical trials and has potent antievaporative action in vitro. The objective of this study was to measure the level of oxygen in PFHO.

Methods: T1 relaxation times (time taken for proton spins to translate from a random alignment to an alignment with the main magnetic field) for fluorine-19 in perfluorohexyloctane were measured using fluorine-19 nuclear magnetic resonance spectroscopy. The level of oxygen was interpolated from published data.

Results: The hydrogen-1 and fluorine-19 nuclear magnetic resonance spectra of PFHO were well resolved and the resonance assignments and intensities were as expected. The T1 values calculated for the CF_3 group resonance in the current study was 0.901 seconds and 1.12 seconds at 25 °C and 37 °C, respectively. The T1 values for the CF_2 group resonances increased by 17% to 24% with an increase in temperature from 25 °C to 37 °C. The mean (SD) partial pressure of oxygen in PFHO was calculated to be 257 (36) mm Hg and 270 (38) mm Hg at 25 °C and 37 °C, respectively.

Conclusions: The current study confirms that PFHO contains a significant amount of oxygen, more so than that calculated for tears in equilibrium with air. Once instilled on the eye, PFHO is not expected to be a barrier to the oxygen necessary for a healthy cornea and may in fact deliver nonreactive oxygen to the cornea to facilitate healing in patients with dry eye disease.

Introduction

Perfluorohexyloctane (PFHO) (see Figure 1) is a novel single component eye drop recently approved by the Food and Drug Administration in the United States for the treatment of dry eye disease.[1–5] Recently, PFHO was shown to be superior to other eye drops in its ability to inhibit evaporation in vitro.[6] In the current study, the potential for PFHO to provide oxygen to the cornea was confirmed. PFHO has been used in drug delivery[7–13] and as an endotamponade.[14]

The most common case of evaporative dry eye disease is Meibomian gland dysfunction (MGD). An unstable tear-film lipid layer leads to a pathological cycle of evaporation, inflammation, and ocular surface damage associated with MGD.[15–17] PFHO may be considered a functional substitute for the tear-film lipid layer in patients with dry eye associated with MGD given treatment with PFHO led to a significant change from baseline in total corneal fluorescein staining and patient dryness score (using a visual analog scale compared with saline treatment[3,18,19]; treatment had also significant effects on visual analog scale score for burning/stinging and other dry eye symptoms,

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https://doi.org/10.1016/j.curtheres.2023.100705

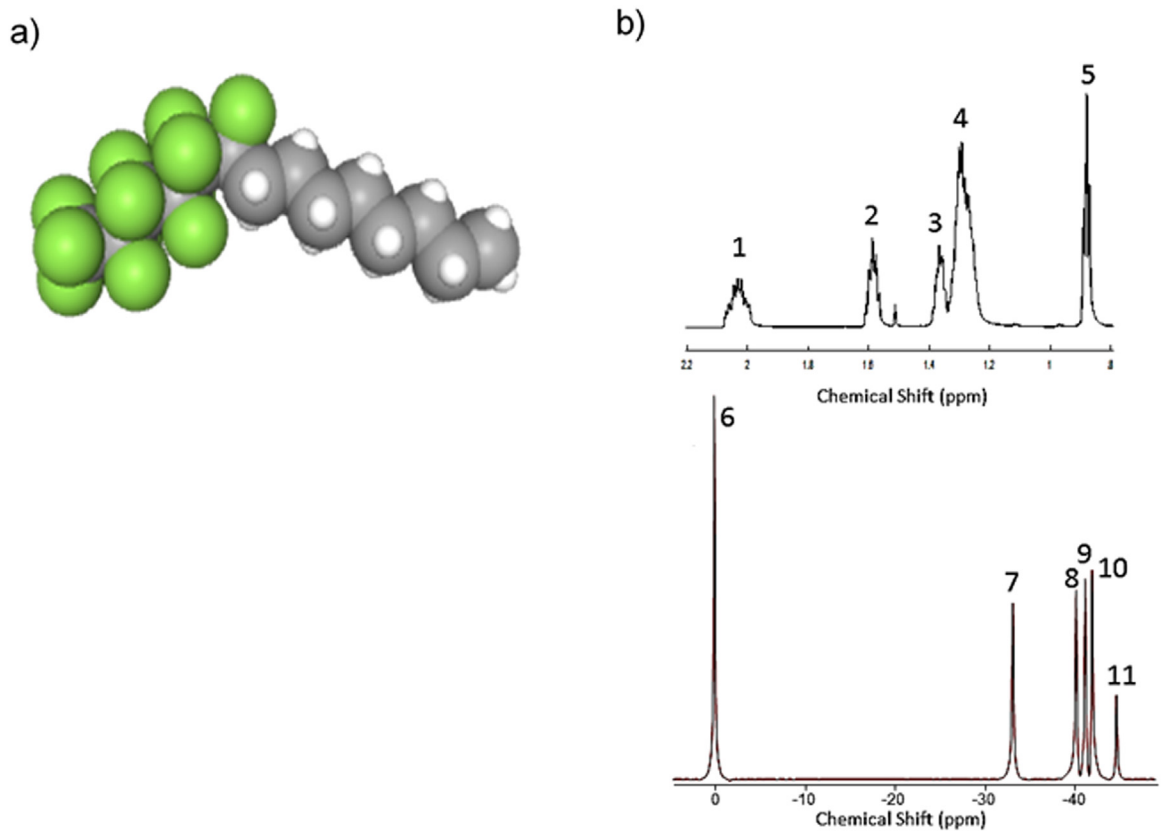

Figure 1. (A) Space filling model of perfluorohexyloctane. White balls are protons, gray balls are carbon atoms, and the green balls are fluorine atoms. Numbers above resonances refer to assignments given in the Table. (B) ^{1}H-nuclear magnetic resonance spectrum of perfluorohexyloctane. (C) ^{19}F-nuclear magnetic resonance spectrum of perfluorohexyloctane.

including foreign body sensation, itching, and sensitivity to light and pain[3,19,20]; its safety[3,18–20]; its optical properties[21]; and its superior ability to inhibit evaporation.[6,21] As a nonaqueous liquid,[6,21] PFHO is likely to reduce surface friction and form a long-lasting antievaporative barrier on the ocular surface facilitating surface healing. PFHO is also biologically, thermally, and chemically stable.[22,23]

Fluorine-19 (^{19}F) is a stable isotope of fluorine exhibiting a nuclear spin allowing it to be studied using ^{19}F-nuclear magnetic resonance spectroscopy (NMR). Seminal ^{19}F-NMR studies published more than 4 decades ago showed that the level of oxygen dissolved in perfluorocarbons is 10 times that of water and that oxygen molecules move freely and rapidly through the perfluorocarbon lattice, associating with PFHO for only 10^{-12} seconds.[24] These properties, in turn, facilitated the development of perfluorocarbons as artificial blood substitutes[23–25] and their use of magnetic resonance imaging to measure real-time tissue oxygenation in vivo.[26–28] The later studies were possible because the spin-lattice relation rate of ^{19}F is dependent on the local partial pressure of oxygen (Po_2). Further, because there are no metabolic or metal-catalyzed oxidation processes occurring in PFHO, one would not expect reactive oxygen species to be present.

The cornea contains no oxygen supplying blood vessels, so oxygen obtained from tears is especially important to corneal health.[29–31] The potential of the novel eye drop, PFHO, to provide oxygen to the cornea was confirmed using ^{19}F-NMR in the current study and implications for its mechanism of action in dry eye disease are discussed.

Materials and Methods

Chemicals

d-chloroform was obtained from Sigma Chemical Co (St Louis, Missouri). PFHO was obtained from Novaliq (Heidelberg Germany).

NMR spectroscopy

Proton hydrogen-1 (^{1}H) NMR of PFHO was acquired on at 700 MHz VNMRS spectrophotometer equipped with a 5-mm ^{1}H{^{13}C/^{15}N} ^{13}C-enhanced cold probe (Agilent Technologies, Santa Clara, California). The PFHO sample used for ^{1}H-NMR contained 50 µL PFHO in 600 µL d-chloroform. Measurement was made with 250 scans, 45^{0} pulse width, a 1.000 second relaxation delay between 0 and 11 ppm. Chemical shifts were referenced to the d-chloroform resonance at 7.25 ppm.

^{19}F-NMR spectral data were acquired on a Inova-500 spectrometer (Varian Associates, Palo Alto, California) using a 5-mm Broad Band coil with a proton decoupler (BBH) probe with the ^{1}H decoupler coil tuned to 470.07 MHz. ^{19}F-NMR PFHO spectra were obtained from a neat (ie, no d-chloroform) sample of PFHO in a single scan. For the purposes of this study, chemical shifts were referenced to the CF_3 group resonance at 0 ppm. T_1 relaxation time measurements were performed by the 180 – τ – 90 inversion-recovery method using the s2pul sequence with the following parameters: 1 scan, 20-second relaxation delay, 100 ppm sweep width, 64K data points and an array of 10 τ values ranging from 0.0125 to 6.4 seconds. To avoid nonlinear pulse width

Table
Assignments for resonances for perfluohexyloctane*

Resonance No.	Chemical shift, ppm	Resonance assignment (red)	T1 Relaxation rate,† sec		Change in T1 with temperature (%)
			25 °C	37 °C	
1	2.03	$-\mathbf{CH_2}-(CF_2)_5$			
2	1.59	$-C\mathbf{H_2}-CH_2-(CF_2-)_5-$			
3	1.36	$\mathbf{-CH_2}-CH_3$			
4	1.28	$-(C\mathbf{H_2})_4-$			
5	0.88	$-C\mathbf{H_3}$			
6	0	$-C\mathbf{F_3}$	0.901 (0.007)	1.12 (0.02)	20
7	33.3	$-C\mathbf{F_2}-\mathbf{CH_2}-$	0.29 (0.02)	0.34 (0.02)	17
8	40.4	$-C\mathbf{F_2}-CF_2-\mathbf{CH_2}-$	0.41 (0.01)	0.500 (0.005)	22
9	41.4	$-C\mathbf{F_2}-(CF_2)_2-\mathbf{CH_2}-$	0.477 (0.004)	0.58 (0.01)	22
10	42.1	$-C\mathbf{F_2}-(CF_2)_3-\mathbf{CH_2}-$	0.395 (0.003)	0.475 (0.008)	22
11	44.8	$-CF_3-C\mathbf{F_2}-$	0.55 (0.01)	0.68 (0.01)	24
Po_2, mm Hg‡		257 (36)	270 (38)		

Po_2 = partial pressure of oxygen.
* Resonances are numbered in Figure 1.
† Values are presented as rate (experimental error).
‡ Interpolated from reference 43 T1 = relaxation time T1.

artifacts over the relatively large spectral window, 2 separate T_1 experiments were performed, one centered on the CF_3 resonance, and the other centered on the 5 CH_2 group of signals.

Commercial software (GRAMS 386; Galactic Industries Corp, Salem, New Hampshire) was used to phase and analyze all NMR spectra. Oxygen concentration in PFHO exposed to air was interpolated from the published linear Po_2—longitudinal relaxation rate plots for PFHO.[43]

Results

The ^{1}H-NMR and ^{19}F-NMR spectra of PFHO were well resolved and the resonance assignments and intensities were as expected (Figure 1 and Table). T1 relaxation times for the ^{19}F resonances are given in the Table. The T1 values calculated for the CF_3 resonance in the current study was 0.901 seconds and 1.12 seconds at 25 °C and 37 °C, respectively. The T1 values for the CF_2 resonances increased by 17% to 24% with an increase in temperature from 25 °C to 37 °C. The mean (SD) Po_2 of PFHO was calculated to be 257 (36) and 270 (38) mm Hg at 25 °C and 37 °C, respectively.

Discussion

The major finding of the current study is that the Po_2 in PFHO is around 260 mm Hg, 68% higher than that published for the surface of the corneal epithelium, or 155 mm Hg, and 3.7 times higher than the Po_2 published for the corneal endothelium and aqueous humor, or 55 mm Hg.[32–34] Our finding is in agreement with previous studies that showed that the oxygen solubility in PFHO is 32.2% to 43.6% by volume.[35,36] The major implication of our finding is that PFHO on the surface of the eye is not a barrier to oxygen necessary for a healthy cornea,[29–31,37–42] but rather, PFHO could serve as a source of oxygen (Figure 2). In this regard, PFHO is superior to commercially available eye drops that are mostly composed of water and likely to contain an equilibrium concentration of oxygen from the air.

T1 is the time constant for the relaxation of the nuclear spin magnetization vector parallel to the external magnetic field. The T1 values calculated for the CF_3 resonance in the current study was 0.901 seconds and 1.12 seconds at 25 °C and 37 °C, respectively, slightly lower but in general agreement, considering the experimental error, with the published T1 values for the CF_3 resonance for PFHO, 1.06 seconds and 1.3 seconds at the same temperatures.[43] We chose to use the CF_3 resonance because its relationship with oxygen levels is well established.[43] The CF_2 resonances for PFHO could also have been used to measure oxygen levels.[44] In the current study, T1 values for all the ^{19}F resonances increased by 17% to 24% with an increase in temperature from 25 °C to 37 °C (Table) in agreement with other studies.[43–46] The ability of T1 to measure oxygen concentration in semifluorinated compounds is because the nuclear relaxation arises from dipolar interactions between the electronic spins of oxygen and the spin of PFHO nuclei. The interaction is modulated by both the translational diffusion of oxygen and PFHO and by the electronic spin relaxation of oxygen.[24]

Oxygen is essential to the cornea, and a lack of oxygen, to as low as 2% to 10% at the surface of the cornea, has been shown to cause corneal swelling.[38–41] It is likely that the lack of oxygen inhibits adenosine triphosphate generation that lowers Na,K-ATPase activity resulting in an increase in corneal hydration and swelling.[47] Whether the eyelid is open or closed, whether the external air is oxygenated or devoid of oxygen, the endothelium and aqueous humor Po_2 is 55 mm Hg.[31–33] Conversely, when the eye is open or closed in the presence of oxygenated air, or when the external air is devoid of oxygen, the epithelium Po_2 is 155, 44, and 0 mm Hg, respectively.[31–33] Consequently, the major source of oxygen for the endothelium is the aqueous humor and vasculature system, whereas the source of oxygen for the epithelium at the corneal surface is the external air via the tears. The current study confirms that PFHO on the surface of the eye is not a barrier to oxygen necessary for a healthy cornea.[28–30,37–42] Oxygen molecules from the air are able to pass through PFHO lattices to tears associating with PFHO for only 10^{-12} seconds.[25]

Oxygen therapy could be beneficial to the cornea. An animal study found that increasing the oxygen concentration in the surrounding environment of the eye could significantly enhance the proliferation ability of corneal epithelial cells and shorten the recovery time after corneal epithelial injury, providing a theoretical basis for corneal epithelial injury in clinics.[47] In humans, hyperbaric oxygen treatment (HBOT) has been used to treat recurrent pterygium,[48] radiation-induced[49] and mitomycin C-induced[50] scleral necrosis, and to treat ischemic diseases of the anterior segment, such as anterior segment necrosis or rubeosis, iridis, or ischemic inner retinal diseases.[50] As an adjunct for scleral buckling procedures in patients with sickling hemoglobinopathies, HBOT prevented anterior segment ischemia[51] and provided significant improvement of visual acuity and visual field in patients with nonarteritic anterior ischemic optic neuropathy.[52] In addition, HBOT temporarily improved visual symptoms in patients with multiple sclerosis.[53]

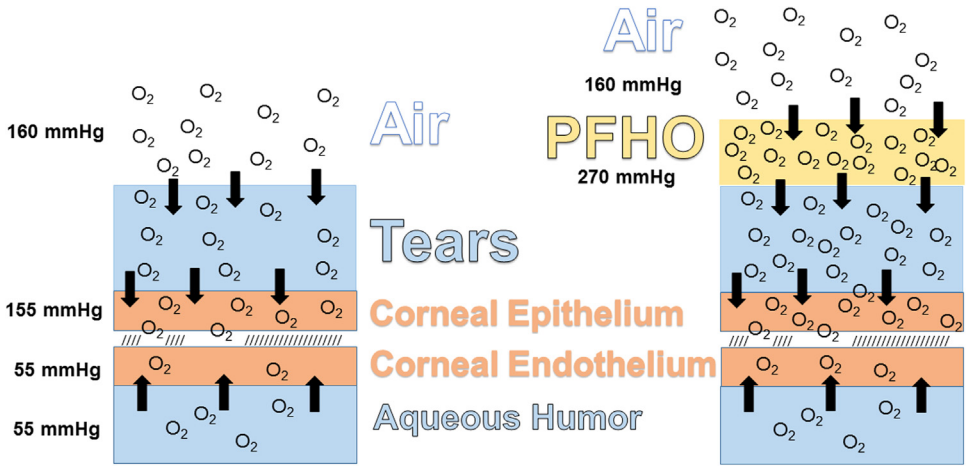

Figure 2. Schematic of oxygen in perfluorohexyloctane (PFHO) layered over tears. The current study shows that the concentration of oxygen in the PFHO layer is much more (270 mm Hg) than that in the air (160 mm Hg). PFHO would be expected to be a source of oxygen necessary for a healthy cornea. PFHO is superior to aqueous eye drops because it is expected to contain much more oxygen.

The safety of HBOT was demonstrated in a study in which no side effects occurred in either patients or animals given 100% oxygen through goggles for 30 minutes.[54] No patient experienced conjunctival injection, corneal edema, or an effect on vision, and no constriction of the iris vasculature was seen.[50] In another study, no patient showed significant acuity or refractive error changes produced by single HBOT sessions. No changes were found in the ultrasound, corneal curvature, or blood analysis data.[39] No changes in the central corneal thickness were observed after 3 months of HBOT.[55] Although HBOT appears to be safe for the cornea, 2 of 8 patients showed significant increases in myopia after 20 2-hour sessions of HBOT,[39] and extended HBOT caused cataracts in guinea pigs.[56] However, HBOT involves oxygen levels that are 3.5 times more than that present in PFHO, and for a longer duration, >20 treatments of 2 hours, so it is unlikely that the oxygen in a drop of PFHO is detrimental to the eye, and indeed is likely to be beneficial as discussed above.

The current study adds support to the positive attributes of PFHO as an eye drop therapy for dry eye disease from other studies that show PFHO is chemically, biologically, and thermally stable[22,23] and as a liquid[21] with a low surface tension[32] spreads over the eye surface, potentially augmenting the tear-film lipid layer.[21] In vitro, PFHO inhibits the rate of evaporation of the underlying aqueous, superior to meibum and other eye drops.[6] It is safe[3,18,19] and effectively reduces symptoms of dry eye.[3,20]

Conclusions

The current study confirms that Po_2 for PFHO is 62% more than that published for air, 68% higher than that at the surface of the corneal epithelium, and 370% higher than the concentration of oxygen in the corneal endothelium and aqueous humor.[31–33] In this regard, PFHO is superior to commercially available eye drops that are mostly composed of water, and at most, contain a concentration of oxygen similar to that of air.

Declaration of Competing Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Acknowledgments

This work was funded by Bausch Health.

Conceptualization, funding acquisition, and supervision were undertaken by Jason Vittitow and Robert Kissling. Data curation, formal analysis, and methodology were undertaken by Neal Stolowich and Douglas Borchman. Investigation, resources, and software were undertaken by Douglas Borchman. Project administration, Jason Vittitow, Robert Kissling, and Douglas Borchman. Writing (review and editing) were undertaken by Neal Stolowich, Jason Vittitow, Robert Kissling, and Douglas Borchman.

The funding source, Bausch + Lomb.

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