Failure of Potassium Siphoning By Müller Cells: A New Hypothesis of Perfluorocarbon Liquid–Induced Retinopathy

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PURPOSE. To determine the effect of perfluorocarbon liquid (PFCL)–induced abolition of potassium siphoning by the vitreal end feet of Müller cells.

METHODS. Porcine eyecups were filled with stained balanced salt solution and PFCLs (perfluorodecalin, perfluoroctane, perfluorohexylhexane, or the semifluorocarbon perfluorohexylhexane). With optical coherence tomography, the distance between PFCL and retina was determined, and the size of the aqueous space covering the retinal surface was estimated. The data were used to calculate the retinal potassium siphoning into small aqueous volumes.

RESULTS. The distance between PFCL and retinal surface was found to be less than 5 to 10 µm with any PFCL tested. The resultant volume of the aqueous space was too small to act as a sufficient sink for K⁺ ion siphoning.

CONCLUSIONS. A certain threshold volume of vitreal fluid seems to be necessary for efficient buffering of intraretinal increases of K⁺ and perhaps other (e.g., H⁺) ions through the Müller cells. When the aqueous fluid is replaced by a PFCL (or by silicone oil) for longer periods, the outer retina becomes subject to long-lasting K⁺ accumulation, and consequent neurodegeneration and reactive gliosis occurs. The authors propose to search for new vitreous-substituting fluids with the capability to dissolve ions. (Invest Ophthalmol Vis Sci. 2000;41:256–261)

In vitreoretinal surgery perfluorocarbon liquids (PFCLs) are used as a mechanical tamponade for complicated retinal detachments, as well as in cases of primary vitrectomy. Because of the high specific gravity of PFCLs, intraoperative stabilization and reattachment of the retina can be facilitated considerably.

Injected as short-term vitreous replacement, these liquids seem to be well tolerated by the retina. Their long-term intraocular tolerance, however, has been questioned. Depending on the type of PFCL and the duration of intraocular retention, several pathologic processes have been described, such as defects of the photoreceptor outer segments, a decreased number of photoreceptor nuclei, intercellular edema of the outer retina, and degeneration of cell processes in the outer plexiform layer. Thus, neuronal degeneration occurs primarily in the outer retinal layers, which are distant from the inner retinal surface exposed to the PFCL. Furthermore, the variations of retinal thickness are accompanied by morphologic changes of retinal Müller (glial) cells. Similar findings have been described after long-term vitreous replacement by silicone oil. It has been hypothesized for the latter case that “the normal exchanges between the retina and the vitreous medium could be impaired.” Such exchanges may involve several kinds of ions and other molecules and are mediated mainly by the Müller cells. Indeed, the vitreal end feet of the Müller cells abut the basal lamina of the internal limiting membrane and therefore are in proximity to the injected PFCL. There is ample experimental evidence that Müller cells buffer the extracellular retinal potassium ion concentration ([K⁺]ₑ) by siphoning excess K⁺ ions into the vitreous humor. Activity-dependent increases of the retinal [K⁺]ₑ have been measured in the inner and outer plexiform layers (at lights-ON and in the subretinal space surrounding the inner and outer segments of photoreceptor cells (at lights-OFF). The Müller cell–mediated spatial buffer mechanism requires that the vitreous act as a sink for K⁺ ions. That is, the vitreous humor must consist of an (aqueous) fluid in which the K⁺ ions can be dissolved and must constitute a large “sink” volume into which many ions can be released before a significant increase in their concentration occurs. Both requirements are easily met by the natural vitreous humor. However, ions do not solve in PFCL. Thus, if there were only small or even no aqueous volumes between a PFCL tamponade and the internal limiting membrane, there would be no sufficient sink for Müller cell–mediated K⁺ fluxes, and large increases of [K⁺]ₑ would occur in the retina, particularly in the outer retinal layers. Such increases, in turn, may induce excitotoxic neuronal degeneration and cell death.

Unfortunately, neither the spatial relationships between a PFCL tamponade and the retina, nor the retinal K⁺ ion fluxes, can be measured in the patient’s eye in situ. Therefore, we
determined the distance between PFCL and retina in excised porcine eyes, and used an established mathematical model\textsuperscript{19} to calculate the spatial buffering $K^+$ currents flowing into the sink volume estimated from the distance measurements.

**MATERIALS AND METHODS**

Porcine eyes were obtained from a local abattoir, transported on ice, and immediately used for the experiments. The anterior segment including the ciliary body was removed with a razor blade, approximately $5$ mm from the limbus. The vitreous was extracted completely with cotton buds; particular care was taken not to touch the retina. Thereafter, the eyecup was rinsed twice with balanced salt solution (BSS). To distinguish the PFCL from the aqueous solution in the vitreous cavity by optical coherence tomography (OCT), the eyecup was filled with a mixture of BSS and $1\%$ Intralipid (Sigma, Deisenhofen, Germany), a $20\%$ suspension of phospholipid-stabilized soybean oil that produces a clearly detectable OCT signal. Then, PFCL ($1$ ml of perfluorodecalin [PFD; Pharm Pur, Augsburg, Germany], perfluorooctane [PFO; Pharm Pur], perfluoroperhexadecfluorooctane [PFH; Fluoron, Neu-Ulm, Germany]) was added.

An experimental OCT device (with approximately $25\mu m$ lateral and $10\mu m$ axial resolution in clinical settings) was used to determine the distance between PFCL and the retina. OCT is similar to ultrasonographic B-scan imaging, except that it uses light rather than acoustic waves. Two-dimensional cross-sectional images of tissue microstructures are constructed from multiple ranging scans of backscatter light versus depth.\textsuperscript{20} To validate the axial resolution within the PFCL–retinal interface, sections of epoxy resin ($5$-, $10$-, and $20\mu m$ thickness) were cut with a microtome and placed on the retina before filling with PFCL.

The measured maximum thickness of the aqueous interspace between PFCL and retina was used to run an established mathematical model of spatial buffering $K^+$ currents through Müller cells.\textsuperscript{19} Because this model was developed for the rabbit retina, and because most of the experimental observations of PFCL-induced retinal degeneration were made in rabbits, the basic parameters of the model\textsuperscript{19} were not changed. Briefly, the model describes passive $K^+$ currents within a “unit tissue cylinder” consisting of one Müller cell surrounded by an extracellular space (ECS) and the adjacent neurons and an overlying aqueous sink volume (Fig. 1A). Under normal conditions, the latter is constituted by the vitreous humor, and can be considered to be indefinitely large.\textsuperscript{19} For the present study, this volume was calculated from the mean cross-sectional area of the unit tissue cylinder (i.e., the surface area of a Müller cell end foot) and the maximum thickness of the aqueous fluid film between the PFCL and the retina, measured in the first series of experiments described earlier. Assuming that during retinal illumination the $[K^+]_o$ within the two plexiform layers increases from $5$ to $6 \text{mM}$ because of neuronal signal processing, $K^+$ currents ($i$ in Fig. 1B), will be driven by the changed transmembrane potentials ($E_i - E_o$ in Fig. 1B) to enter the Müller cell within the plexiform layers, to flow through its cytoplasm, and to leave the Müller cell end foot into the sink volume (for details, see Reference 19).

The amplitude of these currents depends on the extracellular ($R_E$) and intracellular ($R_i$) resistances, as well as the local membrane resistances $R_2$ and $R_4$. Reasonable values for these resistances have been evaluated experimentally or estimated, depending on the geometric properties of rabbit Müller cells.\textsuperscript{19} Under normal conditions, $E_i$ was considered to be constant, because the released $K^+$ ions may easily dissolve within the enormous vitreous volume, and no local $[K^+]_o$ increase would cause a depolarization of the end foot membrane. However, in the case of vitreous replacement by PFCL, the restricted sink volume cannot prevent increases of $[K^+]_o$, and the resultant depolarization of the end foot membrane reduces the driving force for spatial buffering $K^+$ currents. To simulate this effect, our model was modified as a two-capacitor circuit (Fig. 1C). One capacitor ($C_1$), located in the two plexiform layers (which are lumped together for reasons of simplicity), is charged by the excess $K^+$ ions within the extracellular source volume(s) of these two layers. The driving force $E_1 - E_2$ causes a discharging of this capacitor but, as the $K^+$ ions accumulate in the restricted supraretinal sink volume, the second capacitor ($C_2$) at the end foot is charged by the very same current. This must reduce the amplitude of the current and, particularly, the velocity and extent by which a $K^+$ clearance occurs in the outer retina. Using these assumptions and the data explained earlier,\textsuperscript{19} we used the circuit of Figure 1C for a calculation of the time-dependent parameters of $K^+$ current flow in case of vitreous replacement by PFCL.

**RESULTS**

**OCT Imaging of PFCL in Porcine Eyes**

If the injected BSS was labeled with $1\%$ Intralipid, sufficient discrimination was possible between the (nonreflecting) PFCL, the (strongly reflecting) retina, and the (weakly reflecting) labeled BSS (Figs. 2, 3, and 4). First, the axial resolution of our OCT device was determined by placing epoxy resin plates between the retina and the PFCL tamponade. Whereas epoxy resin sections of $5\mu m$ thickness were not easily distinguishable from the retinal surface (Fig. 2A), $10\mu m$ sections were clearly set apart from the surface of the retina (Fig. 2B). Thus, an axial resolution of $5$ to $10\mu m$ was achieved. There were no notable differences among the various PFCLs tested.

When directly applied to smooth retinal areas, all tested PFCLs showed close contact with the retinal surface. Intralipid-labeled BSS was not detectable at the area of contact with the retina, but formed a wedge-shaped space at the sides of the PFCL droplet (Fig. 3). The angle of this wedge-shaped space differed depending on the surface tension, specific weight, and droplet size of the tested PFCL.

In some cases, the experimental procedure caused epiretinal depressions surrounded by retinal folds. These depressions were filled with Intralipid-labeled BSS below the covering PFCL (Fig. 4). In the direct neighborhood of these lesions, the PFCL was in close contact with the retina. Summarizing these data, it may be safely stated that wherever the PFCL contacted the smooth (nonfolded) retina, the aqueous fluid-filled cleft between retina and PFCL had a thickness of less than $5$ to $10\mu m$, at least under our experimental conditions. It remains to be shown whether similar values would be obtained in human ocular surgery; however, we are not aware of a method suitable for measurements in human patients or any obvious reasons to expect significant differences.
FIGURE 1. Modeling of K⁺ siphoning by Müller cells. (A) A retinal tissue cilinder, containing just one Müller cell (yellow) and approximately 15 dependent neurons, is overlayd by a residual aqueous sink volume (green). Between the Müller cell and the neurons, there is an extracellular space (ECS) which, within the two plexiform layers, is loaded with excess K⁺ ions after lights-ON (source volume, red). (B) K⁺ ions enter the Müller cell within the plexiform layers, driven by the membrane potential difference $E_1 - E_2$, flow through the Müller cell stalk, and leave the end foot in the vitreous. The current (i) circuit is completed by balancing Na⁺ and Cl⁻ currents through the ECS and must pass the resistances of the Müller cell membranes ($R_2$ and $R_4$) and the longitudinal resistances $R_1$ and $R_3$. (C) In a further step of abstraction, the current i (still driven by the difference $E_1 - E_2$) flows across the summarized, mainly Müller cell-dependent, resistance $R$ ($R = R_1 + R_2 + R_3 + R_4$) between two capacitors $C_1$ and $C_2$. The capacitor $C_1$ (red) unloads the excess K⁺ ions in the ECS into capacitor $C_2$ (green) which, in turn, is loaded by this process. i has the same amplitude throughout the circuit ([K⁺]source × source volume) = ([K⁺]sink × sink volume). ILM, inner limiting membrane; IPL, inner plexiform layer; OPL, outer plexiform layer; OLM, outer limiting membrane.

FIGURE 2. Determination of the axial resolution of the OCT device through PFCL. Epoxy resin sections (arrowheads) of 5-, 10-, and 20-μm thickness were placed on the retina. (A) Five-micrometer sections were not easily distinguishable from the retinal surface. (B) Ten-micrometer sections were clearly set apart from the level of the retina. PFD, perfluorodecalin; R, retina. The 100-μm scale bar in Figure 4 is valid also for Figure 2.

FIGURE 3. Demonstration of close contact of PFD with the smooth retinal surface (R). The Intralipid-labeled (Sigma) solution (IL) formed a wedge-shaped tongue at the margin of the PFD droplet (arrow) but was not detectable in the area of contact between PFD and retina. The 100-μm scale bar in Figure 4 is valid also for Figure 3.

FIGURE 4. Demonstration of Intralipid-labeled (Sigma) ponds within retinal folds (straight arrows) underlying the PFCL. Note, however, that adjacent to the lesions, PFCL is in close contact with the retinal surface (R). Scale bar, 100 μm.
Mathematical Simulation of K⁺ Siphoning

Thus, for an estimation of the parameters of spatial buffering K⁺ currents from the two plexiform layers into the residual vitreous sink volume, the thickness of the latter was taken as 5 μm. Because PFCL rests mostly on the retinal midperiphery of the lower eye segment, all estimates were made for a midperipheral retinal tissue unit (Fig. 1A) with an average intraretinal volume of 7000 μm³, a height (i.e., retinal thickness) of 120 μm, and a vitreal surface area of approximately 60 μm². 19 This tissue cylinder is overlaid by a residual aqueous sink volume of 60 μm² × 5 μm = 300 μm³. At the other end of the circuit (Fig. 1), there are two source volumes, constituted by the extracellular spaces in the outer (44 μm³) and inner (180 μm³) plexiform layer. 19 Together, the total source volume amounts to approximately 225 μm³ and thus is almost as large as the sink volume.

Assuming that light-flash stimulation would cause an increase of [K⁺]e from 5 to 6 mM in both plexiform layers, the quasi-infinite sink volume of the normal vitreous would “swallow” all the excess K⁺ ions out of the retina within approximately 2 seconds. 19 Thus, shortly after the stimulus, the [K⁺]e in both plexiform layers would be back at the basic level of 5 mM (hatched line in Fig. 5). In the case of vitreous replacement by PFCL, however, the final equilibration of K⁺ ions according to

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[K⁺]_{e\ final} = \frac{[K⁺]_{e\ source} \times V_{source} + [K⁺]_{e\ sink} \times V_{sink}}{V_{source} + V_{sink}}
\]

leaves an increased [K⁺]e of 5.43 mM even at long times after the stimulus (solid line in Fig. 5).

Thus, the thin residual aqueous film under the PFCL was insufficient to support the Müller cell–mediated extracellular K⁺ clearance in the plexiform layers (Fig. 5). This result was not dramatically changed when a thickness of 10 μm rather than 5 μm was assumed for the aqueous film between PFCL and retina. In this case, the final [K⁺]e was approximately 5.3 mM (still well above the baseline of 5.0 mM). It should be pointed out that this simple model simulates the [K⁺]e response to just one light flash, applied to a retina with a normal baseline [K⁺]e. Certainly, further light stimuli (applied to a retina with already elevated [K⁺]e) would cause additive, imperfectly decreasing elevations of [K⁺]e. Unfortunately, these complex changes cannot be simulated satisfactorily, because too many features of the system are unknown. It can safely be concluded, however, that after vitreous replacement by PFCL, long-standing or even steady state [K⁺]e elevations characterize those retinal areas that were overlaid by PFCL for longer periods.

DISCUSSION

PFCLs such as PFD, PFO, and PFP are clinically used as temporary intraocular tools in posterior segment surgery. Usually, they are removed at the end of surgery and are replaced by different vitreous substitutes, such as expanding gases or silicone oils. PFD and PFO have been toxic in animal experiments when left within the eye for extended periods. 1–5,21,22 In the case of humans, long-term intraocular tolerance of PFP has been discussed controversially. 23–24 It is noteworthy that vacuoles in the inner retinal layers were observed by Nabih et al. 24 6 weeks after vitreous replacement with PFP. Viebahn and Buettnner 25 reported a patient with severe ocular intolerance to a PFP tamponade over 5 months in whom epiretinal proliferations developed inferiorly.

It remains uncertain how the PFCL, considered to be inert, can cause the observed pathologic effects in human and animal retinas. Physicochemical and physical (gravity-induced pressure) effects have been implicated. The recently introduced PFCLs with lower specific gravity (e.g., approximately 1.4 g/cm³ or more for the semifluorocarbon PHF) minimize these effects and will provide new insights into the pathogenic mechanism(s).

In the present study, we have tested another hypothesis, based on the assumption that PFCL toxicity is not primarily due to their high specific gravity or possible chemical impurities but to their inability to dissolve ions. 17 Our OCT measurements indicate that PFCLs, including the low specific gravity semifluorocarbon PHF, replace most of the aqueous sink volume available for K⁺ siphoning. Furthermore, our mathematical simulation shows that such small sink volumes are not sufficient to allow a rapid, efficient K⁺ clearance within the retina. Taking into consideration that even small increases of [K⁺]e cause significant depolarizations of the cell membrane, that most retinal neurons have low membrane resting potentials close to the threshold or even the high open-probability range of various voltage-activated ion channels, 26,27 and that excitotoxicity—a vicious circle involving membrane depolarization, release of depolarizing transmitter molecules, and vice versa—is one of the main reasons for neuronal cell degeneration, 18 our results support the idea that impairment of retinal K⁺ clearance may be an important mechanism of PFCL-induced retinal injury. (In regard to depolarization, according to the NERNSTian equation, a [K⁺]e increase from 5 to 5.43 mM causes a depolarization by more than 2 mV, to 6 mM by 5 mV, and so on).

In particular, this hypothesis accounts for the striking observation that whenever retinal changes have been observed after long-lasting PFCL application, they have been localized mainly in the outer retinal layers that are distant from the PFCL droplet. For instance, the [K⁺]e increases in the outer plexiform layer, 14,15,16 normally buffered into the vitreous, 15–15,19,30 may cause the observed local neuronal degeneration 1,2,4,6 if exposure is long standing. Furthermore, if the vitreal pathway is not available for K⁺ buffering currents, the subretinal space may be used as an alternative sink. 10,11 Long-lasting K⁺ accumulations in this space may cause the observed degeneration of photoreceptor cells. 1,2,4,5 Moreover, it should be pointed out that K⁺ ions are not the only kind of extracellular waste products of neuronal activity that are buffered through the Müller cells into the vitreous body. Intraocular changes of extracellular pH have been shown to be buffered by a process called CO₂ siphoning, 33 involving Na⁺/H⁺ exchange between Müller cell end feet and the vitreous. 31,33 A PFCL-induced failure of this exchange may cause intraretinal pH changes that may well contribute to the observed pathologic effects.

Finally, the described failure of spatial buffering may also occur when the vitreous is replaced by silicone oil; in this case, of course, the retina of the upper eye segment should be injured. Indeed, degenerative effects of long-standing silicone
After a rapid increase of the \([K^+]_e\) in both plexiform layers from 5 to 6 mM. Whereas the \([K^+]_e\) goes back to 5 mM within more than 2 seconds when the normal vitreous is present (dotted line), it cannot fall back below 5.43 mM, even after a long delay when the aqueous sink is only 5-µm thick (solid line).

**Figure 5.** Results of the mathematical simulation of \(K^+\) siphoning after a rapid increase of the \([K^+]_e\) in both plexiform layers from 5 to 6 mM. Whereas the \([K^+]_e\) goes back to 5 mM within more than 2 seconds when the normal vitreous is present (dotted line), it cannot fall back below 5.43 mM, even after a long delay when the aqueous sink is only 5-µm thick (solid line).

oil application have been observed in the outer retinal layers of rabbits,\(^8\) monkeys,\(^9\) and humans.\(^32\) When a silicone–fluorosilicone copolymer oil (heavier than water) was applied, a disappearance of the outer plexiform layer and a disorganization of the photoreceptor layer were observed in the inferior rabbit retina.\(^33\)

Another aspect of prolonged PFCL tamponade is the characteristic diseases of Müller cells described by several investigators. Müller cells have been shown to develop features of reactive gliosis including hypertrophy,\(^1,3,3-6\) expression of glial fibrillary acidic protein,\(^34\) and droplike protrusions between the inner segments of the photoreceptors.\(^3\) These changes may represent secondary responses to the neuronal degeneration mentioned earlier. However, Müller cells may be directly injured by the elevated \([K^+]_e.\) Müller cells cultured in high-\(K^+\) media were shown to increase their protein synthesis and even their proliferation rate.\(^35\)

Furthermore, if spatial buffering of \(K^+\) is impaired, Müller cells are stimulated to remove the excess \(K^+\) ions by active uptake through their Na⁺,K⁺ pumps.\(^36,57\) When Müller cells were cultured in elevated \([K^+]_e\) their Na,K-ATPase activity increased.\(^35\) This activation may finally cause functional overload and metabolic exhaustion of the cells.

It remains unclear why some investigators did not observe PFCL-induced retinal degeneration and gliosis. For example, no retinopathy was reported 6 weeks after vitreous replacement with PFDP\(^24\) and after six months under perfluorooctylbromide.\(^38\) According to the hypothesis of impaired spatial buffering, these cases may be accounted for as follows.

First, PFCL-induced retinopathy requires a very close contact between PFCL and the retinal surface. If, for instance, the residual aqueous space has a thickness of only some 100 µm or more, the mathematical simulation shows no significant deviations from the normal case. Such small but sufficient sink ponds may occur after gas vitrectomy or incomplete mechanical vitrectomy, in which small amounts of vitreous cortex may remain on the retinal surface (occasionally detectable as white precipitates after prolonged intraocularly retained PFCL\(^39,40\)) and at places where local retinal folds arise during or after surgery (see Fig. 4). These latter cases may also account for the observation that the retinal damage is not uniform across the injured area.

Second, a PFCL-induced impairment of \(K^+\) buffering probably must last for longer periods before retinal injury occurs. It should be kept in mind that activity-induced (even long-lasting or repetitive) elevations of the retinal \([K^+]_e\) are normal side effects of vision. Thus, if the PFCL bubble is frequently moving and therefore covering a given retinal area for relatively short periods, retinal injuries may be prevented. This may make small bubbles more tolerable than large ones. Moreover, it may explain why in human patients (who expose different parts of the retina when they change the position of the head, e.g., during sleep) less dramatic injuries were described than in rabbits, which maintain an extremely constant position of the head, even when the body is turned around its longitudinal axis, and even during sleep. Such translocations of the silicone oil tamponade in human patients may also account for the observation that ganciclovir is sufficiently released into the retina from implants located in the aqueous meniscus below the silicone oil tamponade.\(^41\)

However, there may be a further reason why PFCL- and silicone oil–induced retinal diseases are mainly observed in rabbits. The midperipheral (i.e., exposed) rabbit retina is essentially avascular.\(^42,43\) whereas the human retina is well vascularized throughout. In avascular retinas, \(K^+\) buffering is exclusively directed to (and dependent on) the vitreal sink, whereas in vascularized retinas, excess \(K^+\) ions may be transferred into retinal capillaries as well as into the vitreous.\(^11\) It is thus possible that the PFCL-induced absence of a vitreal sink is better tolerated by species, such as humans, with a vascularized retina. If so, PFCL-induced injuries would be expected particularly in those human patients in whom the situation is complicated by insufficient retinal blood supply. Indeed, Eckardt and Nicolai\(^6\) reported on hypertrophic Müller cells in a human retina after long-term vitreous replacement with PF. The excised specimen originated from a peripheral retinectomy due to proliferative vitreoretinopathy, probably accompanied by poor circulation.

In summary, the \(K^+\)-siphoning hypothesis of PFCL-induced retinopathy is neither clearly proven nor rejected, but it is certainly supported by our experiments and simulations and by many data from the literature. Assuming that this mechanism indeed contributes significantly to clinical problems, it may be asked what practical consequences we can propose. Apart from the advice to remove the PFCL early in cases of poor retinal circulation, three recommendations may be made: to use small bubbles, and to ask the patient to change the position of his or her head frequently; to perform an incomplete resection of the vitreous intentionally, thus leaving a rather thick cortex of vitreous fluid; and to search for fluids with both a high specific gravity and the ability to dissolve ions. The value of the first two suggestions is certainly limited: The bubble must (continuously) rest on the entire detached area to reattach and stabilize it, and the tractional forces and/or proliferating cells within the vitreal cortex may be the very cause for the treatment, so that this cortex must be removed as completely as possible. Thus, there remains the search for a new type of fluid with ion-dissolving properties, which is highly recommended.
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References