

# Histological Study of Intraocular Changes in Rabbits After Intravitreal Gas Injection

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**Abstract:** To study the effects of different intravitreal gases on intraocular tissues, adult pigmented rabbits were given 0.4 mL intravitreal injections of air, 50% sulfur hexafluoride (SF<sub>6</sub>) and air, 100% SF<sub>6</sub>, 50% perfluoropropane (C<sub>3</sub>F<sub>8</sub>), or 100% C<sub>3</sub>F<sub>8</sub>. On postinjection days 1, 4, 7, and 14, the eyes were removed and the iris, ciliary body and retina processed for light and electron microscopy. Histopathological examination found no abnormalities in eyes that received air and none in the irises of gas-injected eyes. Eyes that received 50% or 100% SF<sub>6</sub>, or 50% C<sub>3</sub>F<sub>8</sub> had vacuolar changes in the ciliary body and retina after maximum gas expansion; these changes were transient, returning to normal as the gas was absorbed. Eyes that received 100% C<sub>3</sub>F<sub>8</sub> suffered irreversible damage to the ciliary body and retina, in both the superior and inferior portions. These observations indicate that expansion of some intravitreal gases can cause both reversible and irreversible changes in intraocular tissues. The degree of damage is affected by the duration of exposure and the gas concentration. Highly concentrated, long-lasting gases can cause irreversible changes resulting in breakdown of the blood–ocular barrier and impaired retinal function. Jpn J Ophthalmol 1997; 41:278–283 © 1997 Japanese Ophthalmological Society

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#### Introduction

Intravitreal gas injection has been widely used for treatment of several vitreoretinal conditions. In 1911,  $Ohm^1$  first used this technique for rhegmatogenous retinal detachment. In 1938, Rosengren<sup>2</sup> reattached a retina with intravitreal air injection combined with diathermy and drainage of subretinal fluid. In 1968, Norton et al<sup>3</sup> noted the advantages for treatment of giant retinal tears. With the development of modern closed vitrectomy in the 1970s and 1980s, long-lasting intravitreal expanding gases such as SF<sub>6</sub> and C<sub>3</sub>F<sub>8</sub> have been used for proliferative vitreoretinopathy.<sup>4,5</sup> In 1986, Rosengren's<sup>2</sup> air injection technique was reintroduced by Hilton and Grizzard<sup>6</sup> as pneumatic retinopexy.

Complications associated with the intravitreal gases include corneal endothelial damage, elevated

Jpn J Ophthalmol 41, 278–283 (1997) © 1997 Japanese Ophthalmological Society Published by Elsevier Science Inc. intraocular pressure, inflammatory reaction, gas cataract, vitreous opacification, vitreous detachment, new retinal tears, macular pucker, and proliferative vitreoretinopathy.<sup>6-15</sup> However, some investigators have suggested that intravitreal gas injections do not result in irreversible retinal dysfunction or histopathological damage.<sup>5,11,16-19</sup> We previously observed that intravitreal gas was associated with breakdown of the blood–ocular barrier, cataract formation, changes in aqueous humor composition, and impaired retinal function.<sup>20–23</sup> This study histologically examined the effects of intravitreal gases on rabbit intraocular tissues.

# **Materials and Methods**

Forty eyes of 40 adult pigmented rabbits weighing 2.5–3.5 kg were used. Rabbits were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrochloride and 2.0 mg/kg of xylazine. After complete mydriasis with 0.5% tropicamide and 0.5% phenylephrine hydrochloride, 0.4 mL air or gas aspirated through a 0.22  $\mu$ m filter (Millipore, Milford, MA, USA)

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**Figure 1.** Electron micrograph: ciliary body, day 4, 100% SF<sub>6</sub>; vacuolar degeneration of NCE; normal ST.



Figure 3. Electron micrograph: ciliary body, day 4, 50%  $C_3F_8$ ; changes in ST collagen fibers and neutrophil infiltration.

was injected intravitreously into the left eye 1 mm from the corneal limbus at the 6 o'clock position, with a 27-gauge needle. Gases used were air, 50% SF<sub>6</sub> with air, 100% SF<sub>6</sub>, 50% C<sub>3</sub>F<sub>8</sub> with air, and 100% C<sub>3</sub>F<sub>8</sub>; each gas was given to eight rabbits. Animals were cared for and handled in accordance with the ARVO statement on use of animals in vision and ophthalmic research.

On days 1, 4, 7, and 14 after injection, the eyes were examined by slit-lamp microscopy and indirect ophthalmoscopy; intraocular pressures were measured by applanation pneumotonography (Alcon Surgical, Fort Worth, TX, USA). Rabbits were sacrificed by intravenous injection of ketamine hydrochloride; eyes were removed and fixed in 4% glutaraldehyde for 2 hours. Half of each eye was used for hematoxylin and eosin staining for light microscopy; the other half was fixed in 1% OsO4, dehydrated, embedded in epoxy resin, and prepared in ultrathin sections for electron microscopy.

#### Results

In all gas-injected eyes, the maximum intraocular pressure occurred immediately after gas injection but decreased within 1 hour and remained normal throughout the 14-day follow-up period.

Since the rabbit vitreous cavity volume is approximately 1.5 mL,<sup>23</sup> the 0.5 mL air bubble filled only one-fourth of the cavity and was gradually absorbed, disappearing completely within 4 days. The 100% SF<sub>6</sub> expanded to its maximum, about double, in 2–3



**Figure 2.** Electron micrograph: ciliary body, day 7, 100% SF<sub>6</sub>; decreased vacuolar NCE degeneration.



**Figure 4.** Electron micrograph: ciliary body, day 4, 100%  $C_3F_8$ ; vacuolar NCE degeneration.



**Figure 5.** Electron micrograph: ciliary body, day 7, 100%  $C_3F_8$ ; vacuolar NCE degeneration.

days, and filled half the cavity. This gas was also absorbed gradually, disappearing in 7–10 days. The 100%  $C_3F_8$  bubble expanded to 3–4 times its volume in 4 days, almost completely filling the vitreous cavity. It was absorbed very slowly, remaining in the vitreous for at least 14 days.

## Iris

No abnormalities were found in any eyes.

#### Ciliary Body

No abnormalities were found in eyes that received air or 50% SF<sub>6</sub> with air. Eyes that received 100% SF<sub>6</sub> had marked vacuolar changes in the nonpigmented epithelium on days 4 and 7, especially in the superior



**Figure 6.** Electron micrograph: ciliary body, day 14, 100%  $C_3F_8$ ; partial recovery of vacuolar NCE degeneration.



**Figure 7.** Electron micrograph: ciliary body, day 14, 100%  $C_3F_8$ ; vacuolar degeneration and cellular infiltration.

portion of the eye. These changes disappeared by day 14 (Figures 1, 2). Eyes that received 50%  $C_3F_8$ showed degeneration of both superior and inferior portions on day 1; changes in collagen fibers, and infiltration of neutrophils were observed by day 4 (Figure 3). These changes decreased by day 7 and tissues were nearly normal by day 14. Eyes with 100%  $C_3F_8$ had marked vacuolar changes in the nonpigmented epithelium of both superior and inferior portions on day 1, increasing in severity by day 4. Vacuolar changes and neutrophil infiltration of both the pigmented and nonpigmented epithelium were seen in both superior and inferior portions on day 7 (Figures 4, 5). These changes resolved partially, although vacuolar changes and cellular infiltration were still present on day 14 (Figures 6, 7).

#### Retina

Light microscopy found no retinal abnormalities in air-injected eyes at any time. Eyes that received 50% SF<sub>6</sub> developed vacuoles in the nerve fiber layer of both superior and inferior portions on day 7; these had returned to normal by day 14 (Figure 8). Mild vacuolar changes in the nerve fiber layer, both superiorly and inferiorly, were produced by day 7 in eyes that received 100% SF<sub>6</sub> and returned to normal by day 14, except for some vacuolar changes visible in the superior retina (Figure 9). Eyes that received 50%  $C_3F_8$  developed vacuoles in the nerve fiber layer on day 4, extending to the outer nuclear layer by day 7 and returning to normal by day 14 (Figure 10). Perfluoropropane at 100% produced vacuoles in the superior nerve fiber layer on day 1, extending to the inner nuclear layer with disruption of the outer



**Figure 8.** Light micrograph: retina, day 7, 50% SF<sub>6</sub>; vacuolar degeneration in nerve fiber layer.

photoreceptor segments. The inferior retina of these eyes was slightly less damaged, with vacuolar changes only in the nerve fiber layer, with outer segment disruption. Destruction of the retinal pigment epithelium was seen on days 4 and 7 (Figure 11).

Electron microscopy of these eyes showed destruction of the retinal pigment epithelium, and inner and outer photoreceptor segments on day 7 (Figure 12). By day 14, the outer layer of the retina had returned to normal, although vacuoles were still present in the nerve fiber layer (Figure 13).

# Discussion

Air and other expanding gases commonly used for vitreoretinal surgery were believed to be free of chemical or pharmacologic toxicity to intraocular tis-



**Figure 10.** Light micrograph: retina, day 7, 50%  $C_3F_8$ ; vacuolar degeneration in outer plexiform layer.

sues. Any harmful effects associated with their use were thought to be related to the physical effects of increased ocular pressure causing glaucoma or retinal occlusion, or prolonged contact with certain ocular tissues causing corneal endothelium damage or cataract. In our rabbit model, however, the elevation of intraocular pressure in all treated eyes was transient, lasting no more than 1 hour, as noted in previous papers.<sup>11,19,20</sup> Elevation of intraocular pressure was also unrelated to the expansion rate or concentration of the gases.

Constable et al<sup>9</sup> first described the breakdown of the blood-ocular barrier after intravitreal gas injection in monkeys. We have found previously that intravitreous gas is associated with an increase in aqueous flare in rabbits.<sup>20</sup> The present study suggests that long-lasting intravitreal gas can damage the pig-



**Figure 9.** Light micrograph: retina, day 7, 100%  $SF_6$ ; vacuolar degeneration in nerve fiber layer.



**Figure 11.** Light micrograph: retina, day 4, 100%  $C_3F_8$ ; destruction of retinal pigment epithelium.



**Figure 12.** Electron micrograph: retina, day 7, 100%  $C_3F_8$ ; changes in retinal pigment epithelium.

mented and nonpigmented epithelium of the ciliary body and the retinal pigmented epithelium, that is, both the blood-aqueous barrier and the blood-retinal barrier.

Previous articles suggest that intravitreal gas injections do not cause retinal dysfunction or irreversible histopathological damage to the retina.5,11,16-19 Finberg et al<sup>16</sup> reported that vitreous replacement with either air or an air/SF<sub>6</sub> mixture in owl monkeys causes localized thickening of the photoreceptor outer segments with irregular arrangement of the disks, and invasion by pigmented macrophage-like cells between the pigment epithelium and the retina. After the gas was absorbed, electroretinography did not show any statistically significant difference in b-wave amplitude and threshold response between normal and SF<sub>6</sub>-injected eyes. Kishimoto et al<sup>19</sup> reported that the intravitreal injection of 0.5 mL air or 100% SF<sub>6</sub> in rabbits caused vacuolar changes in the outer plexiform layer, ganglion cell layer, and retinal pigment epithelium, with irregular arrangement of



**Figure 13.** Electron micrograph: retina, day 14, 100%  $C_3F_8$ ; vacuolar degeneration in nerve fiber layer.

photoreceptor outer segments. These changes were reversible and returned to near normal in 7–14 days with the absorption of the air or  $SF_6$ .

Our present study shows that highly concentrated  $C_3F_8$  can cause reversible damage to the retina. We previously noted that injection of 0.4 mL 100%  $C_3F_8$  in rabbit eyes fully displaces the vitreous at maximum expansion, causing a significant reduction in awave and b-wave amplitudes.<sup>23</sup> These results indicate that intravitreal injection of highly concentrated long-lasting gases can cause irreversible damage to the retina and retinal function.

Avascular ocular tissues such as the cornea and crystalline lens are more susceptible to damage with prolonged gas contact: The avascular rabbit retina may be more vulnerable to the harmful effects of gas injection than the vascular human retina. However, because the retina is often ischemic in patients with proliferative diabetic retinopathy, branch vein occlusion, or chronic retinal detachment, treatment with  $C_3F_8$  gas tamponade may affect retinal function even with an anatomically successful vitrectomy procedure.

It is not known whether the changes we observed are due to chemical, pharmacologic, physical, or toxic effects of the gases.<sup>24</sup> The primary cause may be simple drying of retinal tissues by direct contact with the gas, but the inferior retina in eyes injected with  $SF_6$ and  $C_3F_8$  also showed mild abnormalities. Changes in the vitreous following gas injection, such as altered ion balance and osmotic pressure<sup>25</sup> or gas toxicity, itself, may be a significant factor in retinal damage.

Although all commercial gases have a minimum purity level of 99%, SF<sub>6</sub> may contain sulfur pentafluoride and hydrogen fluoride, and  $C_3F_8$  may contain some halocarbons.<sup>26</sup> Sulfur hexafluoride is known to be toxic to the development of some insects.<sup>27</sup> Because peak damage to intraocular tissues occurs after maximum expansion of the gas, absorption of the gas may cause the retinal damage.

Expanding gas tamponade following vitrectomy has recently been used for the treatment of idiopathic macular holes, but peripheral or inferior visual field depression has occasionally been noted, even with successful closure of the macular hole.<sup>28</sup> Such visual field disturbance is believed to result from surgical damage to the nerve fiber layer during posterior vitreous detachment, or from the direct toxic effects of the intravitreal gases on the retina.

The degree of damage depends on the intravitreal longevity of the gas. An injection of 0.4 mL 100%  $C_3F_8$  (equivalent to total replacement of vitreous volume with 25%  $C_3F_8$ ) caused marked and irreversible damage to the retina and retinal function. The retinal changes seen with 50%  $C_3F_8$  (equal to replacement with 13%  $C_3F_8$ ), and 50% or 100%  $SF_6$  (equal to replacement with 13% or 25%  $SF_6$ ) were all reversible. This suggests that use of lower concentrations of gases may prevent postoperative retinal damage.

Injection of long-lasting intravitreal gas may cause both reversible and irreversible changes in the ciliary body and retina. The severity of the damage depends on the longevity of the gas used; it also appears that peak damage occurs with maximum expansion of the gas. Use of highly concentrated  $C_3F_8$  or repeated injections of gas may contribute to retinal dysfunction, even with anatomically successful vitreous surgery.

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