Retinal Damage by Air Infusion during Vitrectomy in Rabbit Eyes

Tadashi Hasumura, Naoko Yonemura, Akira Hirata, Yasuhiro Murata, and Akira Negi

PurposE. Visual field defects after vitrectomy can be seen after any surgery involving fluid-air exchange. To elucidate the effect during surgery of the infused air on the retina, the present study investigated the changes in the morphology of the rabbit retina induced by air infusion and the changes resulting from varying amounts of infused air pressure.

MEtHods. Eighteen eyes of 18 rabbits were used. A standard three-port vitrectomy with artificial posterior vitreous detachment followed by fluid-air exchange was performed in 12 eyes. During the fluid-air exchange, humidified air was infused with an air pressure of 25 or 40 mm Hg for 30 seconds. As a control, vitrectomy without fluid-air exchange was performed in six eyes. The eyes were enucleated and fixed immediately. Specimens were processed and examined by light and scanning electron microscopy (SEM).

resulTs. With SEM, sharply demarcated retinal lesions were observed at the opposite side from the infusion cannula in all eyes in which a fluid-air exchange was performed. At the lesion, the internal limiting membrane was often detached, and the underlying nerve fiber layer was exposed. Light microscopy revealed that the inner retina was most affected, with concomitant swelling of the inner plexiform layer and the inner granular layer. In addition, the retina was often focally detached with adhesion of some retinal pigment epithelial cells to the photoreceptor cells. Increased infused air pressure was accompanied by a significant increase in the area of retinal damage. In contrast, no morphologic change was observed in the control eyes.

Conclusions. Air infusion during vitrectomy can cause mechanical retinal damage in the rabbit retina. The mechanical damage may result in a visual field defect after vitrectomy. (Invest Ophthalmol Vis Sci. 2000;41:4300–4304)

A postoperative visual field defect is one of the serious complications possible after vitrectomy. After the first description of a visual field defect after vitrectomy by Melberg and Thomas in 1995,1 many clinical reports have proposed hypotheses about the causes of this complication, such as mechanical trauma to the optic nerve head, damage to the optic disc by artificial posterior vitreous detachment, disturbance of choroidal circulation, retinal damage by a gas bubble, and elevation of postoperative intraocular pressure.2–4 However, the exact cause has not been clarified. Recently, some investigators have noted that the location of the postoperative visual field defect was affected by changing the location of the infusion cannula.5,6 This result proposed that the infusion of air during fluid-air exchange affects the retina and causes this complication.

Clinically, we have experienced that the location of the visual field defect is strongly affected by the location of the infusion cannula and that reducing the infused air pressure decreases the incidence of the complication.7 Based on these results, we hypothesized that the main cause of this complication is mechanical retinal damage from the air infusion during and after fluid-air exchange. To confirm this hypothesis, we investigated the changes in the morphology of the rabbit retina induced by air infusion during surgery. We analyzed the changes resulting from different levels of infused air pressure.

METHODS

Animals

Eighteen eyes of 18 Japanese adult albino rabbits (Kyudo, Kumamoto, Japan), 12 weeks of age and weighing 2.0 to 2.5 kg, were used in this study. The eyes were examined by means of indirect ophthalmoscopy to exclude the presence of pre-existing fundus abnormality. Animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the Committee on Animal Research of Kumamoto University.

Surgical Procedures

The rabbits were anesthetized with pentobarbital (20 mg/kg intravenously, Nembutal; Dainippon Pharmaceuticals, Osaka, Japan) and ketamine hydrochloride (20 mg/kg intramuscularly,
Ketalar 50; Sankyo Pharmaceuticals, Tokyo, Japan), after pupils were maximally dilated with a mixture of 0.5% tropicamide, 0.5% phenylephrine hydrochloride, and 1% atropine sulfate. After the administration of topical drops of 2% lidocaine hydrochloride, a conjunctival flap was made at the supertemporal side. A sclerotomy was made at approximately 3 mm from the corneal limbus for placement of the infusion cannula (20-gauge, 4 mm in length) which was connected to the ophthalmic irrigation solution (Ope-guard MA; Senju Pharmaceuticals, Osaka, Japan). Two additional ports were made at the pars plana to introduce the vitreous cutter and light source probe. Using a vitreous cutter (Ocutome 8000; CooperVision Systems, Irvine, CA), lensectomy, core vitrectomy, and artificial posterior vitreous detachment (PVD) were performed under a surgical microscope.

Animals were divided into three groups, depending on the surgical procedures performed. Six eyes underwent surgery without any further manipulations as control subjects. Another 12 eyes underwent fluid-air exchange with the use of an automatic insufflation regulator (PS-100; Takata CooperVision, Tokyo, Japan). After the fluid-air exchange, one side port was kept open for 30 seconds to introduce the air freely into the vitreous cavity under the pressure of 25 mm Hg in six eyes (group A) or 40 mm Hg in the remaining six eyes (group B). The infused air was humidified as described previously by Ohji et al. After the air infusion, the side ports were immediately plugged with scleral plugs. The side ports and the infusion port were then sutured and closed with two to three bites of running shoelace suture. The time from the beginning of fluid-air exchange to closure of all side ports averaged 3.5 minutes, which was nearly equal to the time needed for the equivalent procedure in a clinical situation. After the surgery, animals were killed with an overdose of intravenous injection of pentobarbital. The eyes were enucleated immediately and injected with a fixative of 2.5% glutaraldehyde and 2% paraformaldehyde mixture in 0.1 M phosphate buffer into the vitreous cavity at room temperature for 10 minutes. The eyes were cut circumferentially at the limbus to make posterior cups and then immersed in the fixative for an additional hour. Two eyes of each group were studied under light microscopy (LM), and the remaining four eyes of each group were studied by scanning electron microscopy (SEM).

**Specimen Preparation for SEM**

The specimens were immersed in 2% tannic acid (Wako, Osaka, Japan) overnight at room temperature to increase tissue reactivity with osmium tetroxide, rinsed with distilled water for 2 hours, and then fixed with 1% osmium tetroxide for 2 hours at 4°C. The specimens were dehydrated in a graded ethanol series, infiltrated in 100% t-butanol, frozen, freeze dried by evaporation in a vacuum, mounted on aluminum stubs, and gold coated. They were observed at an accelerating voltage of 15 to 20 kV with a scanning electron microscope (JSM 6400FK, JEOL, Tokyo, Japan).

**Specimen Preparation for LM**

The specimens were rinsed with distilled water for 30 minutes, dehydrated in a graded ethanol and xylene series, and embedded in paraffin. Sections 2 to 4 μm thick were stained with hematoxylin and eosin (H-E) and examined.

**Infused Air Volume Measurement**

Air infusion was performed with a side port open as described, and the infused air volume was measured using a flowmeter (Floline; STEC, Kyoto, Japan). Values were presented as means ± SD. Mean air flow velocity was calculated as the total air-flow volume divided by the area of the infusion cannula.

**Morphometric Measurement**

Measurements of structural changes in the retinas were made on SEM photographs (magnification, ×50 to ×100) with computer software (NIH Image, National Institutes of Health, Bethesda, MD). Values are presented as means ± SD. Differences among groups were analyzed by the Kruskal–Wallis test. Scheffé’s test was also used as a multiple-comparison posttest. Differences were considered significant when \( P < 0.05 \).

**RESULTS**

**Macroscopic Findings**

During the surgery, the retinas in the control animals had smooth surfaces in whole areas. The retinas in groups A and B, however, exhibited focal, oval-shaped, and whitened changes with serous retinal detachment at the opposite side from the infusion cannula.

**SEM Observation**

Figure 1 shows a retinal surface observed by SEM. In the control group, whole areas of the retina appeared smooth. Fine grooves extending from the optic disc to the periphery, consistent with underlying nerve fiber bundles, were observed at higher magnification (Fig. 1A). The vitreous fibers were completely removed except in the region of the medullary rays (Fig. 1A).

In contrast, the retinas in group A, in which air infusion was performed with pressure of 25 mm Hg, exhibited focal irregular internal limiting membranes (ILM), observed at the side opposite the infusion cannula. In these areas, normal fine grooves became faint. Concentrically arranged ridges of the retinal surface were frequently observed (Fig. 1B). However, the remaining areas of the retina appeared smooth, similar to that in the control group. In group B, irregularity of the ILM was more prominent, exhibiting a dark speckled pattern (Figs. 1C, 2A). In addition, at the center of the lesion, the ILM was detached, and the underlying end feet of Müller cells were observed in all four eyes (Fig. 2B). Furthermore, in three of four eyes in group B, the nerve fiber layer was exposed (Figs. 1C, 2C), which indicated profound damage to the retina. Except for the lesions described, other areas of the retina appeared smooth and normal.

For statistical comparison among groups, the areas of the retinal lesion were evaluated according to the degree of retinal damage (Figs. 2A, 2B, 2C), irregularity of ILM (Figs. 2A, 2D), detachment of ILM (Figs. 2A, 2B, 2D), and exposed nerve fiber layer (Figs. 2C, 2D). There were no detectable lesions in the control group (Fig. 3). In group A, the retinas showed 0.45 mm² of ILM irregularity and 0.05 mm² of detachment of ILM (Fig. 2B). The retinas in group B demonstrated more severe retinal damage with 1.91 mm² of ILM irregularity, 0.67 mm² of detachment of ILM, and 0.23 mm² of centrally located, exposed nerve fiber layer. In all detected areas of the lesions,
there were significant differences in each of the three types of retinal damage among groups ($P = 0.006$, $P = 0.009$, and $P = 0.028$, respectively). In group B in particular, the areas of ILM irregularity and detachment of ILM were significantly larger than those in the control group ($P = 0.002$ and $P = 0.003$, respectively) and significantly larger than those in group A ($P = 0.015$ and $P = 0.004$, respectively; Fig. 3).

**LM Observation**

Figure 4 showed a histologic transverse section of the retina and choroid in the eyes of the control group (Fig. 4A) and in groups A (Fig. 4B) and B (Fig. 4C) at the areas of the retina opposite side from the infusion cannula, which corresponded with the lesion obtained from SEM observation. The retina in
the control group exhibited a regularly layered arrangement. No histologic changes were observed (Fig. 4A). In contrast, the retinas in group A showed a swollen and thicker inner plexiform layer with an increase of approximately 70% and a distended inner nuclear layer (Fig. 4B). Although the outer retina consisting of the outer plexiform layer, outer nuclear layer, and photoreceptor layer appeared unchanged, maintaining its constant thickness and normal arrangement, the retina was often separated from the retinal pigment epithelium (RPE) with a few pigment granules adhering to the outer segment of the photoreceptor cells (Fig. 4B). However, the area adjacent to the lesion appeared normal in its arrangement and thickness.

Furthermore, the retina in group B exhibited detachment of the ILM and nerve fiber layer (Fig. 4C). The arrangement of the outer retina appeared to show less damage, but the retina was prominently detached with numerous RPE cells attached to the photoreceptor cells.

**Infused Air Volume**

The air volume infused by opening of a side port for 30 seconds in Group A was $100 \pm 7$ ml. The air-flow velocity at the tip of the infusion tube in group A was $11.8$ m/sec. In contrast, the air volume infused in group B was $169 \pm 6$ ml, which was significantly higher than that in group A ($P < 0.0001$, unpaired t-test). The air-flow velocity in group B was $19.9$ m/sec, which was 69% higher than that in group A.

**DISCUSSION**

In this study, we observed the morphologic changes occurring in the rabbit retina after fluid-air exchange. The results...
showed that infused air caused focal retinal damage such as irregularity in the ILM, detachment of the ILM, and exposure of underlying Müller cells and nerve fiber bundles. In addition, the finding of retinal detachment with pigment granules adhering to the outer segments of the photoreceptor cells suggests acute mechanical stress in the retina.

The rabbit retina is thinner (90–160 μm) than the human retina (100–560 μm), and the rabbit retina may therefore be more susceptible to physical stress. A preliminary experiment showed that retinal tears or retinal detachment could occur easily in the rabbit retina with during infusion of air at 50 mm Hg. Based on that finding, we chose to use an infused air pressure of 25 and 40 mm Hg in this study instead of the 30 and 50 mm Hg normally used in clinical evaluation.

Hutton et al. reported that clinical patients with postoperative visual field defects had a corresponding profound loss of nerve fiber thickness. Electrophysiological study using a multifocal electroretinogram showed that damage to the inner retinal layer could cause postoperative visual field defects. Studies support our own findings that inner retinal damage is a main pathologic change after vitrectomy with fluid-air exchange.

In contrast, other reports postulated the disturbance of the retinal or choroidal circulation, which indicates potential damage to the outer retina. The present study also showed a certain amount of separation between the photoreceptors and RPE. In addition, RPE cells were detached from the basal lamina and attached to the outer segment of the photoreceptor cells. This result may indicate that the infusion of air not only causes direct retinal damage in the inner retina but also affects the outer retina by inducing sudden retinal separation from the RPE through shear stress. Clinically, we have found that the retina exhibits subretinal fibrosis and RPE atrophy at the area of the retina corresponding to the location of the visual field defect 6 months or more after surgery (N. Yonemura et al., unpublished data, 2000). Therefore, morphologic changes in the retina that occur in a prolonged period after vitrectomy should be clarified further. In addition, the peeling of the ILM has been widely used clinically for resolution of persistent macular hole, for treatment of macular edema of diabetic retinopathy, or for retinal vein occlusion. Postoperative visual function after ILM peeling should be carefully reviewed, although the procedure affects only the inner retina, whereas ILM detachment by air infusion affects both inner and outer retina.

Welch suggested that the dehydration of the retina was the main cause of postoperative visual field defects. Some researchers have found that the use of humidified air prevents this complication completely. Patel et al. postulated that retinal damage secondary to air infusion could be caused by either of two mechanisms: actual mechanical damage by the air infusion or dehydration of the retina from the air infusion. Our clinical study showed that humidifying the infused air was insufficient for the complete prevention of visual field defects. The present study also indicates that retinal damage can be produced with humidified air. Our results indicate that humidification of the infused air is not the only factor in the prevention of retinal damage resulting in postoperative disturbance of visual function.

In the present study, decreasing the infused air pressure from 40 to 25 mm Hg reduced the area and magnitude of retinal damage significantly. We had already noted that decreasing the infused air pressure from 50 to 30 mm Hg was effective in decreasing the incidence of postoperative visual field defects. Decreasing infused air pressure from 40 to 25 mm Hg also reduced the infused air volume from 338 to 200 ml/min. The air flow velocity at the tip of the infusion cannula was also reduced significantly. We believe, therefore, that our hypothesis was correct, that complications produced by mechanical retinal damage are induced by high-pressure infusion of air. Concomitantly, our results also elucidated the efficacy of lowering the infused air pressure during vitrectomy.

In conclusion, we stress the importance of preventing direct mechanical stress to the retina caused by the infusion of air during vitrectomy, because such stress can lead to retinal damage and visual field defects. Decreasing the air pressure is a crucial technique as is using humidified air to decrease mechanical retinal damage. In addition, we are currently developing an instrument for diffusing the infused air, which may be effective in further reducing the risk of the postoperative visual field defects.

References