Retinal Changes after Retinal Translocation Surgery with Scleral Imbrication in Dog Eyes

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PURPOSE. To examine retinal changes induced by scleral imbrication during retinal translocation surgery in dog eyes.

METHODS. Fifteen dogs were anesthetized and underwent retinal translocation surgery. After lensectomy and vitrectomy, an intentional retinal detachment was created, and the upper temporal sclera around the equator was imbricated with five mattress sutures. Translocated distances were calculated by pre- and postoperative photographs. At 1, 2, and 4 weeks after the surgery, the retina was studied by TdT-dNTP terminal nick-end labeling (TUNEL) and immunohistochemistry of peanut agglutinin (PNA) lectin and glial fibrillary acidic protein (GFAP).

RESULTS. The retina was translocated by a mean distance of 0.53 ± 0.30 disc diameters or 959 ± 543 μm. Retinal folds were created around the optic disc in all eyes. Histologic examination of the retinal folds 1 week after the surgery showed many TUNEL-positive cells in the outer nuclear layer, loss of photoreceptor cells, and shortening of the outer and inner segments. A strong immunoreactivity to GFAP was detected in the folds of the retina.

CONCLUSIONS. The results demonstrated that retinal translocation surgery by scleral imbrication inevitably caused retinal folds as a postoperative complication, and the retina within the folds showed extensive loss of photoreceptor cells. It is recommended that the foveal translocation surgery be planned to avoid involving the fovea in the retinal folds. (Invest Ophthalmol Vis Sci. 2000;41:i288–i292)

The neovascular maculopathies, including age-related macular degeneration (AMD), are a leading cause of blindness in elderly people in developed countries.¹,² Several treatment modalities have been used for neovascular maculopathies; however, no satisfying results have been obtained.³–¹⁵ Among the different treatment modalities, the surgical procedure of translocating the fovea onto relatively healthy retinal pigment epithelium (RPE) has achieved some clinical success, measured by an improvement in visual acuity.⁷–¹⁵

In 1993, Machemer and Steinhorst⁷ first performed macular translocation surgery with total peripheral retinotomy. However, proliferative vitreoretinopathy occurred in two of the three patients. Recently, de Juan et al.⁹ developed a new technique of limited macular translocation surgery by scleral shortening or imbrication. Several studies have compared the clinical results of foveal translocation surgeries by partial or total retinotomy with that by scleral imbrication.⁷–¹⁵

The scleral imbrication technique has some advantages because a retinotomy is not required, and there is less surgical trauma. However, the scleral imbrication also has some limitation, such as the limited distance of foveal translocation, the inevitable formation of retinal folds, and the induced corneal astigmatism. Retinal folds involving the fovea are a severe postoperative complication and result in less recovery of visual acuity.¹³

Experimental retinal detachment in animal models has been extensively studied.¹⁶–¹⁹ Prolonged retinal detachment causes degeneration of the photoreceptor cells and apoptosis in the retina.¹⁶,¹⁸ After surgical reattachment of the retina, there is a rapid regeneration of the outer segments of photoreceptor cells within 1 month.¹⁶ However, it is not fully known how much damage occurs in the retina and in the retinal folds after an intentional retinal detachment and reattachment. These retinal changes must be determined to allow surgeons to know the indications and prognosis for foveal translocation surgery.

In this study, we performed retinal translocation surgery by scleral imbrication in dog eyes and evaluated the retinal damages by determining the presence of photoreceptor cells dying by apoptosis and by immunohistochemical staining for photoreceptor cells and glial cells.

MATERIAL AND METHODS

Retinal Translocation Surgery

Fifteen dogs were anesthetized with an intravenous injection of ketamine (5 mg/kg) and an intraperitoneal injection of pentobarbital (10 mg/kg). Atropine was injected intramuscularly. After fundus photographs were taken (Topcon camera;
Tokyo, Japan), the eyes were operated on under an operating microscope (Topcon).

The conjunctiva was cut for 270° with microscissors, and the sclera was exposed. Five mattress sutures were placed on the upper temporal sclera between the superior and inferior rectus muscles at distances of 7 and 11 mm from the limbus. The width of each mattress suture was 4 mm. Scleral resection was not performed. An infusion port was set at 12 o'clock at the limbus, and two sclerotomy sites were created 4 mm from the limbus at 2 and 10 o'clock. After lensectomy, the anterior and posterior lens capsules were removed with a vitreous cutter, and core vitrectomy was performed. A posterior vitreous detachment was created with a soft-tipped needle, and a peripheral vitrectomy was performed. An intentional retinal detachment was then created by irrigating balanced salt solution (BSS) into the subretinal space with a 30-gauge needle. To obtain total retinal detachment, fluid–air exchange was used to force the subretinal fluid into the posterior pole. After a total retinal detachment had been created, the scleral shortening was performed by tightening the five mattress sutures. The detached retina was then reattached by fluid–air exchange, and retinal folds were pushed aside using a back-flush needle. Laser photocoagulation was not performed around the retinal holes in the posterior pole. The air was exchanged by 20% SF6 gas, which formed a tamponade pressing the retina against the choroid. The sclerotomy sites were closed, and the conjunctiva was sutured.

Dexamethazone was injected into the subconjunctival space, and an antibiotic ointment was instilled in the cul-de-sac. After the operation, an antibiotic was injected intramuscularly. The procedures were monitored with a CCD camera (3 CCD; Elmo, Nagoya, Japan) and video recorder. The surgically prepared eyes were observed with an indirect ophthalmoscope periodically.

The animals were killed at 1 hour and 1, 2, and 4 weeks after the surgery. Only one eye was operated on in each animal. Three eyes were obtained at 1 hour, and 4 eyes each at 1, 2, and 4 weeks after the surgery. All procedures involving the animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

To determine the translocated distance, we compared preoperative and postoperative video photographs.

Histochemical Procedures

After the dogs were killed by an intravenous overdose of pentobarbital under deep anesthesia, the eyes were removed and fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate-buffered saline (PBS) at 4°C overnight. The anterior segment was removed, and the eyecup was photographed under a biomicroscope. The eyecup was then cut into small pieces, and some were processed for standard histology and the remainder for cryosections.

For standard histology, the tissues were dehydrated through a graded series of ethanol, and after the tissues were placed in two changes of propylene oxide for 15 minutes each, they were infiltrated overnight in a 1:1 solution of propylene oxide and epon epoxy resin (Quetol 812, Nishshin EM, Tokyo, Japan). The pieces were then embedded in 100% Quetol 812, and 1-μm sections were cut and stained with 0.5% toluidine blue and 0.25% sodium borate. The sections were observed and photographed under a microscope (Olympus, Tokyo, Japan).

The other tissues were immersed in 30% sucrose in 0.1 M PBS at 4°C overnight and embedded in optimal cutting temperature compound (OCT; Miles, Elkhart, IN). Cryosections of 10 μm thickness were cut and processed for immunohistochemistry.

Immunohistochemistry

Cryosections were processed for staining with peanut agglutinin (PNA) lectin (Honen, Tokyo, Japan) or anti-human glial fibrillary acidic protein (GFAP) antibody (ICN Pharmaceuticals, Aurora, OH). After the sections were rehydrated in 0.1 M PBS, they were incubated with fluorescein isothiocyanate (FITC)-conjugated PNA lectin (1:500 diluted in 0.1 M PBS) for 1 hour at 37°C. After sections were rinsed in 0.1 M PBS several times, they were mounted and observed under an epifluorescence microscope (Olympus).

For GFAP staining, the sections were incubated with anti-GFAP antibody (1:50 diluted in 0.1 M PBS) for 1 hour at 37°C. After they were rinsed in 0.1 M PBS several times, they were incubated with FITC-conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch, West Grove, PA) for 1 hour at 37°C. They were again rinsed several times in 0.1 M PBS, mounted, and observed.

TUNEL Staining

Cryosections of the retinas taken at 1, 2, and 4 weeks after the surgery were processed to detect DNA fragmentation in the retina by the terminal deoxynucleotidyl-transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) method with a peroxidase kit (ApopTag; Oncor, Gaithersburg, MD) according to the manufacturer’s instructions. Briefly, residues of digoxigenin-nucleotide were catalytically added by TdT to the 5'-OH ends of double- or single-strand DNA. The labeling product was visualized using a diaminobenzidine (DAB)-enhanced commercial method (HistoMark Orange; Kirkegaard & Perry; Gaithersburg, MD), which resulted in reddish-orange positive staining. The sections were counterstained with 1% methyl green.

RESULTS

Retinal Translocation Surgery

The retina was successfully translocated inferiorly in all 15 eyes. At the end of the surgery, retinal folds were detected in the posterior retina between the optic disc and the temporal retina in all eyes by ophthalmoscopy. Only one eye showed development of a localized retinal detachment in the inferior retina 1 week after the surgery; however, the posterior retina was reattached. This eye was removed at that time and processed for histologic examinations. After the eyes were fixed, the anterior part of the eye was removed, and the remaining eyecup was examined under a biomicroscope. A biomicroscopic photograph of a typical retina 2 weeks after the surgery is shown in Figure 1a. Large retinal folds can be seen to run across the posterior retina (arrows). This pattern of retinal folds was similar in all eyes. A magnified photograph of the same region is shown in Figure 1b. In addition to the large retinal folds, smaller folds were visible to offset the redundant retina caused by the scleral imbrication.

The translocated distance of the retina (in disc diameters) just after the surgery was calculated from photographs of seven eyes that were recorded by the video recorder. The mean
The distance of the translocated retina was $0.53 \pm 0.30$ disc diameters (mean $\pm$ SD). This corresponds to a mean movement of $959 \pm 543$ mm.

**Light Microscopy**

All eyes were processed for histology and examined by light microscopy. One of the retinal folds is shown in Figure 2. The architecture of the retina that was reattached to the RPE after the surgery appeared normal, even 4 weeks after the surgery. In contrast, the photoreceptor layer was absent, and there was thinning of the outer nuclear layer (ONL) in the retinal regions involved in the folds, and thus the retina did not reattach to the RPE. These changes were observed in eyes even at 1 week after the surgery, and the ONL was thinner. With increased time, there was a greater loss of photoreceptors and a thinning of the ONL in the region of the folds. Other retinal layers showed no apparent changes by light microscopy after the surgery.
**TUNEL Assay**

A TUNEL assay was performed on cryosectioned retinas. Many TUNEL-positive cells were detected in the ONL, but only in the retinal folds 1 week after the surgery (Fig. 3). The retina outside the retinal folds, which was reattached to the RPE, did not show any TUNEL positivity in the ONL at any time point. The TUNEL-positive cells were detected not only in the ONL but also in the inner nuclear layer (INL) in the retinal folds.

**Immunohistochemistry**

Cryosections of the retina were stained with FITC-conjugated PNA lectin. In normal retinas, the PNA-stained cone matrix sheath extended for the full length of the photoreceptor layer. In the retinal regions involved in the folds, the PNA-positive staining of the cone matrix sheaths were short (arrows in Fig. 4); however, the retina properly reattached on the RPE showed normal length of PNA-positive staining.

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**DISCUSSION**

Foveal translocation surgery is a promising treatment for neovascular maculopathy. Although limited in number and follow-up period, the patients with AMD or myopic neovascular maculopathy who have undergone macular translocation surgery by scleral shortening have shown excellent improvement of visual acuity.\(^9\)\(^{13}\) The advantages of this macular translocation surgery are: retinotomy not required, shorter operating time, less surgical trauma to the retina and the eye, and low occurrence of proliferative vitreoretinopathy. The disadvantages are the limited translocated distances, unpredictability of the location of the translocated fovea, and corneal astigmatism.

In previous studies, the translocated distances induced by scleral imbrication were 114 to 1919 \(\mu\)m in humans.\(^9\)\(^{11}\)\(^{13}\) In this study, the average translocation distance of the retina was 0.53 \(\pm\) 0.3 disc diameters, which corresponds to approximately 959 \(\pm\) 543 \(\mu\)m. The dimension of adult canine eyes used in this study is only slightly different from that of human eyes: an axial length of approximately 24 mm and a large corneal diameter of approximately 13 mm. However, the translocated distances are comparable to those reported in the
clinical studies. The surgical procedures were similar to those used in humans, and the pattern of retinal folds was always very similar, because the optic disc was not moved, and the redundant retina was held in place at the optic disc. Therefore, whenever scleral imbrication was created in the upper temporal sclera and the retina was intentionally moved inferiorly, retinal folds were always created from the disc edge to the inferior retina and sometimes involved the macular area.

Retinal folds involving the fovea have been reported in patients who have undergone limited macular translocation surgery. However, the retinal folds may be directed with intraocular air by adjusting the position of the face and head after the surgery. In any case, some other designs of scleral imbrication should be developed to reduce the risk of foveal involvement in the retinal folds. In our study corneal astigmatism was not measured; however, the eyeball was obviously deformed immediately after the scleral imbrication.

Histology of the retina after the surgery showed that the retina was normal where it had reattached to the RPE, but the retina involved in the folds showed loss of photoreceptor cells. Previously, retinal folds were created after retinal reattachment surgery for experimental giant retinal tears and demonstrated cystoid retinal edema and long outer segments of photoreceptor cells. Posterior retinal folds involving the macula have been reported as a postoperative complication after retinal detachment surgeries. These posterior retinal folds caused metamorphopsia and decreased visual acuity, and were similar to the retinal folds observed in the present study. In this study, TUNEL assay, and PNA lectin staining demonstrated that damage to the photoreceptor cells and loss of the photoreceptor cells in the retinal folds occurred partly through apoptosis. In contrast, the retina reattached to the RPE were not TUNEL positive. The results of previous studies and our study indicate that care should be taken not to involve the macula in the retinal fold, because visual acuity will be affected.

There was an increase in GFAP positivity over the entire retina after the translocation surgery, indicating the stress induced in the whole retina by the surgery. However, the stress was stronger at the bottom of the retinal folds. It should be noted that an increase in GFAP immunoreactivity in the retina has been induced by vitrectomy alone, and thus this response may be induced easily in the retina.

In conclusion, retinal folds have been shown to be harmful to the retina, as indicated by the loss of the photoreceptors. Unfortunately, those folds cannot be avoided in macular translocation surgery by circumferential scleral imbrication. Our findings indicate that we should be cautious to avoid involving the macula in the retinal folds and to improve our surgical techniques and postoperative care, such as face positioning, after limited macular translocation surgery.

References