Histological changes in the healing process of sclerotomy sites after 20-gauge and transconjunctival 23- and 25-gauge incisions in experimental model*

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Aim: To evaluate the healing mechanism and histological changes of the 20-gauge standard, and 23- and 25-gauge transconjunctival sutureless vitrectomy (TSV) incisions in rabbits.

Materials and methods: Twelve of 16 albino rabbits’ right eyes underwent sclerotomy of either 20-gauge standard, or 23-gauge or 25-gauge TSV incisions. Four rabbits were tested as controls. The study eyes were examined clinically on the first postoperative day and in 3-day periods for 2 weeks. After 2 weeks, rabbits were euthanized; globes were enucleated and underwent histological examination. The sclerotomy sites were investigated histopathologically under light microscope for wound healing semicantitatively.

Results: Pathological sections were examined to monitor the healing course of the sclerotomy sites. Edema, mononuclear inflammatory cell infiltration, fibroplasia, foreign body reaction, neovascularization, and reactive-regenerative changes on ciliary body were evaluated and scored. Fibroplasia and reactive-regenerative changes on ciliary body were statistically significant in eyes treated with 20-gauge standard sclerotomy (P < 0.05). For foreign body reaction results, there was no statistically significant difference between all groups (P > 0.05).

Conclusion: Conventional 20-gauge sclerotomies required conjunctival peritomy and suturing. Transconjunctival 23-gauge and 25-gauge sclerotomy incision procedures without sutures and peritomy decrease overall surgical time, avoid the local inflammatory reaction to the suture materials, decrease postoperative inflammation, and support rapid healing recovery.

Key words: Sclerotomy, transconjunctival sutureless vitrectomy incision, wound healing

Deneysel hayvan modelinde 20-gauge ve transkonjonktival 23- ve 25-gauge sklerotomi sahalarının iyileşme sürecindeki histolojik değişiklikler

Amaç: Tavşanlarda; 20-gauge sütürlü, 23-gauge ve 25-gauge transkonjonktival sütürsüz yapılan sklerotomilerin yara yeri iyileşmesinin değerlendirilmesi amaçlanmaktadır.

Histological changes in the healing process of sclerotomy

Introduction

Through traditional pars plana vitrectomies, sclerotomies are opened with a 20-gauge micro vitreoretinal (MVR) blade and, at the end of the surgery, they are sutured. In recent years, sutureless microincision vitreoretinal surgery has been introduced as an advantageous technique that provides reduced surgical trauma, less postoperative inflammatory response, rapid postoperative wound healing, and more rapid recovery time (1-3). Chen et al. (4) described a new technique, namely self-sealing pars plana sclerotomy, and other surgeons modified this method (2,5). Fujii et al. (6) developed a 25-gauge transconjunctival vitrectomy in 2002 and this increased the popularity of sutureless microincision vitreoretinal surgery. During microincision surgery, sclerotomies are not sutured because of their small size and presumed self-sealing properties. Furthermore, no conjunctival suturing is required at the end of the surgery as sclerotomies are performed through the conjunctiva without conjunctival dissection.

A 25-gauge transconjunctival sutureless vitrectomy (TSV) system had some disadvantages, such as decreased flow rates, flexible instrumentation, and several complications, such as wound leak, hypotony, and retinal detachment (6). A 23-gauge TSV was developed in 2005 as an alternative to 25-gauge vitrectomy (7). Although the technique has its advantages over the traditional 20-gauge sutured incisions, there have been concerns regarding the safety of the sutureless nature of these incisions (8,9). Hypotony, endophthalmitis, and choroidal hemorrhage have been cited as postoperative complications. Compromised wound integrity is considered to be an important factor in these complications of ocular surgery (10).

Although there exist several clinical and experimental studies about various methods of vitreoretinal surgery, there are few studies regarding the wound healing of these sclerotomy sites. The aim of this study was to evaluate the histological changes in the healing process of sclerotomy sites after 20-gauge standard and 23- and 25-gauge TSV incisions in rabbits.

Materials and methods

Sixteen New Zealand white rabbits of both sexes weighing approximately 2 to 4 kg each were used in this study. All animals were handled in accordance with the Animal Care and Use Committee and the Association for Research in Vision and Ophthalmology. The experimental protocol was approved by the Committee of Animal Use and Care of Dokuz Eylül University, School of Medicine. The rabbits were housed under 12/12 h light-dark cycle and allowed free access to water and food. Four of the 16 rabbits were used as controls. Twelve rabbits were assigned to 3 groups (n = 4 in each group) in terms of 20, 23, and 25-gauge sclerotomies (20-gauge: 1.15 mm, 23-gauge: 0.72 mm, and 25-gauge: 0.5 mm wide). The right eyes were used as study eyes. Before the sclerotomies, the rabbits were anesthetized by an intramuscular combination of ketamine hydrochloride (30 mg/kg) and xylazine hydrochloride (6 mg /kg). The pupils were dilated with 2.5% phenylephrine hydrochloride and 10% tropicamide. After ocular surface anesthesia was administered with a topical instillation of proparacaine hydrochloride, the eyes were washed with several drops of 5% povidone iodide.
The 23- and 25-gauge sclerotomies consisted of a 1-step transconjunctival cannula insertion using a beveled trocar (Alcon, Fort Worth, TX, USA) at 2 mm posterior to the limbus of the rabbits’ right eyes, avoiding the lens and the peripheral retina. For angled incisions, the conjunctiva was laterally displaced with the aid of forceps or a Q-tip. The eye was penetrated as tangentially as possible parallel (30°) to the limbus with the bevel up. Once past the trocar sleeve, the angle was changed to 90° (perpendicular to the globe). The cannulas were removed by pulling them from the collar trying to follow the oblique entry. The vitrectomies were performed with movements to mimic physiologic pars plana vitrectomy with ocular manipulation for 5 min. At the end of the vitrectomy, the infusion fluid flowed freely from the superotemporal and superonasal cannulas indicating the absence of vitreous at the wound site. The superonasal and superotemporal cannulas were then removed with the infusion running and immediately covered with a sterile cotton-tipped applicator. Each sclerotomy site was examined for leakage indicated by the formation of a subconjunctival fluid bleb. If leakage was noted, further pressure was applied with the cotton-tip applicator to the site until leakage ceased. The infusion was then turned off, and the infusion cannula was removed and immediately covered with a cotton-tipped applicator.

Conjunctival peritomy was performed before 20-gauge sclerotomy. The sclerotomies were made horizontally with a 20-gauge MVR blade, 2 mm posterior to the limbus. The vitrectomies were performed as described above. At the end of the vitrectomy, sutures were put at 3 sclerotomy sites (8.0 vicryl) and conjunctiva (8.0 vicryl).

Tobramycin 0.3%, dexamethasone sodium phosphate 0.1%, and 10% tropicamide were applied into the inferior conjunctival fornices 3 times a day through the postoperative day 7. The study eyes were examined clinically on the first postoperative day and in 3-day periods for 2 weeks. After 2 weeks, the rabbits were euthanized by an intravenous injection of an overdose of sodium pentobarbital (80 mg/kg body weight). The eyes were enucleated with careful manipulation to preserve globe integrity. Each eye was immediately placed and fixed in 10% formaldehyde solution for 24 h for light microscopy. Gross sectioning was performed after fixation. During dissection, the superotemporal sclerotomy site was identified. A surgical marking pen was used prior to creating each incision to help confirm the entry location. Appropriate tissue specimens containing the incisions were processed routinely for light microscopy. Transverse sections of globes were cut in sclerotomy areas. The specimens were routinely-processed and embedded in paraffin. Thin sections (5 μm) were stained with hematoxylin and eosin. Under a light microscope (Nikon Eclipse E200), sclerotomy sites were investigated semiquantitatively as mild, moderate, and severe for wound healing. Edema, mononuclear (lymphocytic) inflammatory cell infiltration, fibroplasia, foreign body reaction, neovascularization, and reactive-regenerative changes on ciliary body were evaluated and scored.

Digital photos of each histological slide were taken. NIH Image software (version 1.62, National Institutes of Health, Bethesda, MD, USA) was used for analysis of both gross and histological photos.

Statistical analysis was performed with SPSS software version 11 (SPSS Inc., Chicago, IL, USA). Mann-Whitney U and chi-square tests were used in statistical analyses. Mann-Whitney U test was used for fibroplasia, neovascularization, and reactive-regenerative changes on ciliary body scores and chi-square test for foreign body reaction.

Statistical significance was considered when the $P$ value was ≤0.05.

Results

There were no intraoperative complications during the vitrectomy in any eyes. A cotton-wool-tipped applicator was used to gently massage the entry site after cannula removal. All cannulas remained in place with no bleb formation visualized after removal of any of the cannulas. There was also no leakage in the 20-gauge group after the surgery. At the end of all procedures, fundus examinations revealed no abnormalities, such as vitreous hemorrhage or retinal detachment. During the follow-up period (2 weeks), there was no inflammatory response, the lens and vitreous appeared clear and the fundus intact in all study eyes.
The sclerotomy sites were investigated histopathologically and edema, mononuclear (lymphocytic) inflammatory cell infiltration, fibroplasia, foreign body reaction, neovascularization, and reactive-regenerative changes on the ciliary body were evaluated and scored. These findings were summarized in the Table. Mann-Whitney U test was used for fibroplasia, neovascularization and reactive-regenerative changes on ciliary body scores and chi-square test for foreign body reaction.

There was no statistically significant difference between all groups for edema, mononuclear inflammatory cell infiltration, and foreign body reaction ($P > 0.05$). A statistically significant difference was noted between control group (Figure 1) and all other groups for fibroplasia ($P < 0.05$). There was a statistically significant difference between 20-gauge group and all other groups for neovascularization ($P < 0.05$). There was a statistically significant difference between all groups except between 23-gauge and 25-gauge group for reactive-regenerative changes on the ciliary body ($P$

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Edema</th>
<th>Mononuclear inflammatory cell</th>
<th>Fibroplasia</th>
<th>Foreign body reaction</th>
<th>Neovascularization</th>
<th>Reactive changes on ciliary body</th>
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<tr>
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<td>-</td>
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<tr>
<td>Control 4</td>
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<td>-</td>
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<tr>
<td>20G-1</td>
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<td>Moderate</td>
<td>Severe</td>
<td>+</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
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<td>Mild</td>
<td>Moderate</td>
<td>-</td>
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<tr>
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<td>+</td>
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<td>-</td>
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<td>Severe</td>
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<td>+</td>
<td>-</td>
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Figure 1. Note the control section (Hematoxylin & eosin, ×10, original magnification) (Red arrow: Corneal epithelium; Black arrow: Ciliary body).
< 0.05). Inflammatory wound healing was noted in the 20-gauge group (Figures 2a and 2b). Fibroplasia and reactive-regenerative changes on the ciliary body were statistically significant in the 20-gauge group (P < 0.05) compared with both 23-gauge and 25-gauge groups (Figures 3a and 3b). Outcomes were similar for 23-gauge and 25-gauge angled incisions.

Figure 2a, 2b. (20-gauge) Note the moderate mononuclear cell infiltration and severe fibroplasias, foreign body reaction and scattered histiocytic cells (Hematoxylin & eosin, ×10, original magnification) (Blue arrow: Conjunctival epithelium; Red circles: Multinuclear giant cell (Foreign body type); Black circle: Vertical section of suture material; Black arrow: Horizontal section of suture material) (Figure 2a); Higher magnification of black circle (Figure 2b).

Figure 3a, 3b. (25-gauge section) Mild edema, moderate mononuclear cell infiltration and moderate fibroplasia and chronic granulation tissue formation (Hematoxylin & eosin, ×10, original magnification) (Blue circle: Fibrosis; Red arrow: Vessel section; Black arrow: Lymphocytic cells) (Figure 3a), Higher magnification of Figure 3a (Figure 3b).
Discussion

Three-port 20-gauge vitrectomy systems have been the gold standard for vitreous surgery since 1974 (11). During the past 30 years, its instrumentation has successfully been developed further. Conventional 20-gauge sclerotomies required a conjunctival peritomy and suturing. Conjunctival peritomy might be a risk factor for glaucoma and limbal stem cell surgeries in terms of inflammation and adhesions. In contrast to conventional 20-gauge vitrectomy, 23-gauge and 25-gauge transconjunctival sutureless sclerotomy procedures do not require sutures and peritomy decreases overall surgical time, avoids the local inflammatory reaction to the suture materials, and decreases postoperative inflammation and disruption of conjunctiva and sclera (1,2,12). The vitrectomy incision construction using the trocar and cannula method in the TSV facilitates easy entry, with minimal trauma to the conjunctiva, sclera, and the pars plana (13). Throughout the surgery, all instrument shafts pass through the sleeve of the cannula. This minimizes tissue manipulation and microtrauma due to repeated insertion and removal of instruments (6,14). Reduction in the incision size in various surgical procedures has led to tissue trauma minimization and reduction in postoperative convalescence period. This is due to reduced inflammation, less pain, and faster healing.

Keshavamurthy et al. (1) reported differences in sclerotomies undertaken with 20- gauge and 25-gauge instruments in the same patient. Ultrasound biomicroscopy of the sclerotomy sites was performed in the same patient in whom both 20-gauge and 25-gauge sclerotomies had to be constructed during pars plana vitrectomy and the differences were studied. They concluded that healing of a 25-gauge sclerotomy is expectedly quite rapid, with inability to detect the site of sclerotomy in a short duration of 2 weeks postoperatively. This is as opposed to conventional sclerotomies, which might take up to 6-8 weeks postoperatively for complete opposition.

In TSV, incision type is also an important issue. The importance of creating oblique incisions as opposed to straight incisions was highlighted in several recent in vitro studies where angled incisions were superior grossly, histopathologically, and with anterior segment optical coherence tomography (OCT) (15,16). Singh et al. (15) demonstrated ingress of ocular surface fluid (India ink) into sutureless straight incisions following 25-gauge vitrectomy in cadaver human globes. Taban et al. (16) evaluated the dynamic morphology and integrity of sutureless vitrectomy wounds (23-gauge and 25-gauge) in intact cadaver rabbit eyes (with overlying conjunctiva) using anterior segment OCT and histologic analysis with India ink. OCT revealed that straight incisions gaped considerably more than oblique incisions. It demonstrated open wounds with straight incisions under all IOP conditions, with a slightly larger wound width under high IOP conditions. They suggested that oblique incisions provide better wound apposition and stability compared to straight incisions and therefore pose certain implications. Oblique incisions are thought to help decrease or prevent wound leakage, by having an internal lip that presses against the outer lip through intraocular pressure, thereby helping to close the wound (17-21).

We undertook this study to analyze the histological structure of conventional 20-gauge and sutureless 23-gauge and 25-gauge oblique vitrectomy incisions. Before the onset of healing and complete wound closure of a sutureless wound, the wound architecture can be expected to influence the outcome of the surgery, such as postoperative hypotony and postoperative endophthalmitis. Regarding our microscopical findings, fibroplasia and reactive-regenerative changes on the ciliary body were statistically significant in eyes that were treated with 20-gauge standard sclerotomy. For foreign body reaction results, there was no statistically significant difference between all groups. Compared with 20-gauge vitrectomy, histopathological findings in the areas of 25-gauge and 23-gauge transconjunctival sutureless sclerotomy have lighter postinflammatory reactions and fibroplasia. Our histopathological findings are in accordance with similar studies of the healing mechanism and histological changes of sclerotomy sites after 20-gauge standard, and 23- and 25-gauge transconjunctival sutureless sclerotomy (22,23). These findings may indicate that mild inflammatory response and mild degree of fibroplasias are associated with 23- and 25-gauge transconjunctival sutureless sclerotomy. There are certain inevitable drawbacks of this study. It is unclear whether the rigidity of the rabbit scleral fibers is more
than that of human scleral fibers. This may have affected the physiologic outcomes, but would likely have little bearing on the anatomic outcomes. It can also be expected that as the time of the vitrectomy increases or excess manipulation occurs at sclerotomy sites, there may be greater amounts of physiologic leakage and insult to scleral tissues. Our study utilized a 5-min timed vitrectomy as this is the usual time required for complete removal of rabbit vitreous.

In this study we investigated the wound healing course of the rabbit's sclerotomy. Histopathological findings in the areas of 25- and 23-gauge transconjunctival sutureless sclerotomy have lighter postinflammatory reactions and fibroplasias than 20-gauge vitrectomy. These findings support that 23- and 25-gauge sutureless vitrectomy techniques yield lower reactive inflammatory tissue changes that might affect wound healing positively.

References