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Histopathologic Comparison of Conventional Radial Keratotomy and Minimally Invasive Radial Keratotomy in Rabbits

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Abstract: The aim of the present study was to compare conventional radial keratotomy (RK) with minimally invasive RK (mini-RK) in terms of achieved incisional depth as well as the histopathologic changes in the rabbit corneal structures. Four conventional RK incisions were performed on the right eye and four mini-RK incisions were performed on the left eye of 12 Island rabbits using a centripetal cutting technique. The corneas were excised 20 days after the procedure and examined by light microscopy. Histopathologic examination showed that the mean achieved incisional depth (73.47%) in conventional RK was consistent with the intended incisional depth (80%). However, the mean achieved incisional depth (47.28%) was far from the intended incisional depth (80%) in eyes receiving mini-RK. The difference between achieved incisional depth of the two surgical techniques was statistically significant ($t = 10.70$, $P < 0.05$). Corneal structural changes and epithelial plug formations were less in eyes in mini-RK than in conventional RK. These findings suggested that the refractive results in mini-RK may be less effective than conventional RK. On the other hand, in the mini-RK group, less epithelial plug formation and limited histopathologic structural alterations may have an important role in preventing long-term overcorrection and corneal rupture after ocular trauma demonstrated in conventional RK technique. **Jpn J Ophthalmol 1997;41:269–273** © 1997 Japanese Ophthalmological Society

Key Words: Histopathologic changes, hyperopic shift, mini-RK, refractive surgery.

Introduction

Radial keratotomy (RK) was first described by Sato[1] in 1953, and then popularized by Fyodorov in 1974.[2] Refractive surgery is becoming more widespread these days. For this reason, numerous refractive procedures have been advocated in the past 10 years.[3] However, RK is still one of the most effective refractive procedures for correcting myopia and is believed to have a place today, as well as in the future, for certain diopters of myopia.[4]

The aim of radial incisions is to induce the steepening of the peripheral cornea and the flattening of the central cornea, resulting in a reduction in refractive power. Although conventional RK has been declared a safe procedure, there are still some concerns about its safety and predictability.[5] The main concern with RK is the long-term corneal instability and hyperopic shift, which are the most significant refractive complications of radial keratotomy. Minimally invasive radial keratotomy (Mini-RK) was developed by Lindstrom[6] in order to minimize the long-term complications, reduce the invasiveness, and also maintain the benefits of radial keratotomy.

This study is a histological comparison of the mini-RK incision technique and the conventional RK technique in terms of achieved incisional depth and invasiveness in vivo. To the best of our knowledge, this is the first histopathologic comparative study of conventional RK and mini-RK.

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Materials and Methods

Twelve young, non-pigmented, locally available Island rabbits, weighing 2–3 kg, were used in this study. The experimental animals were anesthetized with intramuscular xylazine (5–10 mg/kg, Rompun, Eczac*i*baş*i*, Istanbul), and ketamine hydrochloride (30–40 mg/kg, Ketalar®, Parke-Davis, Morris Plains, NJ, USA). Topical 0.4% oxibuprocaine (Benoxinate®) was also applied to the cornea prior to surgery. Additional doses were given as needed.

Paracentral corneal thickness at the 3-mm optical zone was measured at four points (temporal, inferior, nasal, superior) using an ultrasonic pachymeter (Mentor/Teknar Ophthasonic, St Louis, MO, USA). A central optical zone at the 3-mm point was then marked on the cornea by an optical zone marker through the operating microscope (OPMI:99 Microscope, Carl Zeiss, Germany). The diamond blade was set at 80% of the thinnest paracentral region for all animals. The temporal quadrant was frequently found to be the thinnest area. Four centripetal radial incisions were then made, using a Russian front-cutting RK blade, from the limbus to the 3-mm central optical zone in the right eye, and from the 7-mm optical zone to the 3-mm central optical zone in the left eye of the rabbits. All pachymetric measurements and incisions were performed by the same surgeon (HE). After surgery, 0.5 ccs of gentamicin sulfate was injected subconjunctivally. The animals were sacrificed with an intravenous overdose of thiopentone sodium 20 days after the surgery. Their eyes were enucleated and the corneal buttons were immediately fixed with 4% formaldehyde. Following gross inspection, the corneas were divided into 4 strips (approximately 2 × 6 mm) using razor blades and cut perpendicular to the length of each RK incision. This way, each strip contained one incision. A total of 96 incisions in 24 eyes were examined (four RK incisions per eye). Four serial sections were made perpendicular to each incision at an interval of 70 μm along the wound bed to determine the shape and depth of the incision. The tissues were then dehydrated in a graded series of alcohol, and embedded in paraffin. Sections 6 μm in thickness were stained with hematoxylin and eosin and examined by light microscopy.

The incision depths were measured from the center of the incision by light microscope using the micrometer eyepiece and recorded as a micrometer/percent of total corneal thickness. All achieved depth values given on Tables 1–3 are for the temporal site of the corneas of all eyes. Our definition of the incisional wound gape is the distance from the cut edges of the corneal stroma to the corneal epithelium.

The results were analyzed with the Student's t-test to compare the differences between the two incisional methods. A P-value of <0.05 was considered significant.

Results

A mean achieved incisional depth for each eye was calculated by averaging the data of each of the four sections separately. There was no statistically significant difference among the incision depths because we used a uniform incision technique for all animals in both incision groups.

Analyses of the incisional depth in conventional RK eyes are shown in Table 1. The average mean preoperative pachymetric reading was 375.00 μm (SD =

Table 1. Preoperative and Postoperative Results in Conventional RK Eyes

Rabbit	Mean Pachymetric Depth in Micrometers	Intended Depth in Micrometers	Achieved Depth in Micrometers[a]
1	410	328 (80%)	311 (75.8%)
2	395	316 (80%)	289 (73.1%)
3	360	308 (80%)	266 (73.8%)
4	365	292 (80%)	263 (72.0%)
5	380	304 (80%)	230 (60.5%)
6	385	308 (80%)	252 (65.4%)
7	375	300 (80%)	304 (81.0%)
8	365	292 (80%)	289 (79.1%)
9	360	288 (80%)	274 (76.1%)
10	375	300 (80%)	283 (78.6%)
11	360	288 (80%)	268 (72.4%)
12	370	296 (80%)	273 (73.7%)
Average	375.0 (SD = 15.52)	301.67 (SD = 11.99)	275.17 (SD = 22.21)

[a]Determined by light microscopy.

Table 2. Preoperative and Postoperative Results in Mini-RK Eyes

Rabbit	Mean Pachymetric Depth in Micrometers	Intended Depth in Micrometers	Achieved Depth in Micrometers[a]
1	395	316 (80%)	206 (52.1%)
2	360	288 (80%)	144 (40.0%)
3	360	288 (80%)	198 (55.0%)
4	360	288 (80%)	202 (56.1%)
5	385	308 (80%)	154 (40.0%)
6	387	309 (80%)	167 (43.1%)
7	380	304 (80%)	176 (46.3%)
8	385	308 (80%)	169 (43.8%)
9	390	312 (80%)	179 (47.1%)
10	380	304 (80%)	183 (48.1%)
11	385	308 (80%)	191 (49.6%)
12	375	300 (80%)	173 (46.1%)
Average	378.50 (SD = 12.23)	302.75 (SD = 9.75)	178.50 (SD = 18.86)

[a]Determined by light microscopy.

15.52); the average intended depth in these eyes was 301.67 μm (SD = 11.99; 80% of preoperative reading) whereas the mean achieved incisional depth was 275.17 μm (SD = 22.21). The latter equals 73.47% of the mean preoperative pachymetric reading.

Analyses in mini-RK eyes are shown in Table 2. The average mean preoperative pachymetric reading was 378.50 μm (SD = 12.23); the average intended depth was 302.75 μm (SD = 9.75; 80% of preoperative reading), while the mean achieved depth was 178.50 μm (SD = 18.86). This finding also equals 47.28% of the preoperative pachymetric reading. There was a statistically significant difference in the achieved depth between the two groups (t = 10.70, $P < 0.05$).

Histopathologic findings observed in conventional RK eyes were epithelial irregular thickening, damage in basement membrane (BM), incisional wound gape reaching the deep stroma, activated keratocytes, and an epithelial plug filling the cavity. Some degenerated epithelial cells at the base of the epithelial plug and mucoid degeneration surrounding the epithelial plug were also noticed. No inflammatory cells were observed (Figure 1).

Histopathologic findings observed in mini-RK eyes were moderate epithelial thickening, damage in BM, incisional wound gape reaching to the middle of the stroma, activated keratocytes, and an epithelial plug filling the wound gape in all eyes and intraepithelial BM in only four eyes (Figure 2). Degeneration of the epithelial cells and mucoid degeneration were minimal when compared to conventional RK-eyes (Figure 3). No changes in the endothelium and Descemet's membrane were determined in either group. No effects from the radial keratotomy incision were apparent in adjacent regions in any eyes (Table 3).

Discussion

Radial keratotomy is still one of the most effective methods for the correction of myopia. Although laser surgery has a promising future, RK is believed to have a place in the future, too.[7] However, long-term refractive stability and the risk of traumatic rupture of the keratotomy incisions appear to be an issue of concern with conventional RK because the normal process of stromal wound healing and remodeling

Figure 1. Conventional RK microphotography showing 75.8% incision depth (rabbit 1, right eye). Note that irregular and increased epithelial thickness, an epithelial plug, fills gape. Mucoid degeneration (small arrows) surrounds epithelial plug. Activated keratocytes (large arrows) are evident at the base of wound. Hematoxylin and eosin, ×100.

Figure 2. Light micrograph showing intraepithelial basement membrane (arrows) in radial mini-RK incisions. Hematoxylin and eosin, ×100.

Figure 3. Light micrograph of mini-RK eye (rabbit 1, left eye) showing 52.1% corneal incision, moderate epithelial thickness, and epithelial plug filling cavity. Hematoxylin and eosin, ×100.

phases continue for many years after RK before a stable configuration is achieved.[8] Patients who have undergone RK are at increased risk of corneal rupture after blunt ocular trauma.[9] Experimental studies have shown a decreased corneal resistance to rupture after conventional RK.[10,11] There have been reports of cases of corneal perforation up to 10 years following severe blunt trauma.[12,13] In addition, a significant percentage of progressive hyperopic shift is encountered in patients following surgery.[14] Corneal instability is a key to RK predictability.[15] The PERK[16] study showed a change in refraction of +1 diopter or more in 43% of the patients at 10-year follow-up. The histologic alterations in corneal structures play a role in this differentiation. The epithelial plugs are frequently one of the major causes of overcorrections.[17] The presence of an epithelial plug in a fully healed keratotomy incision also creates a concentration of stress at the incision site that may predispose the cornea to rupture.[18] Reducing or eliminating this stress would prevent hyperopic shift. In this animal study, the epithelial plug seen in conventional RK eyes filled the wound gapes entirely. However, this finding was much less frequent in mini-RK eyes. Thus, mini-RK might be a safer procedure for patients with high risk for ocular trauma.[19] Pinheiro et al[19] showed that mini-RK may not significantly weaken an intact cornea. They found that the average rupture pressure for the conventional RK group was significantly lower than the average rupture pressure for the mini-RK group. Pinheiro et al[19] also demonstrated that the corneas subjected to mini-RK ruptured at significantly higher pressures than corneas that had undergone conventional RK. The presence of intraepithelial basal membrane formation was observed in eyes with mini-RK. There has been no previous report of this formation occurring in either human or animal RK eyes. It might be a newly formed basement membrane and might represent a deposition of fibrous tissue after an early fibrotic response. However, we are unable to explain exactly why it forms especially in mini-RK eyes and not in conventional RK eyes. Thus, further studies are required for determining the cause and consequences of this formation (Figure 2). Another find-

Table 3. Histopathologic Alterations in Both Conventional RK and Mini-RK Eyes

Technique	Epithelium	Basement Membrane	Stroma	Endothelium and Descemet's Membrane
Conventional RK	Irregular Thickening	Damaged	Deep Stromal Wound Gape, Activated Keratocytes	Normal
Mini-RK	Moderate Thickening, Intraepithelial Basement Membrane	Damaged	Middle Stromal Wound Gape, Activated Keratocytes	Normal

ing was mucoid degeneration, detected especially in conventional RK-eyes; it may be a reaction to the epithelial plug formation.

In order to minimize all these disadvantages, the mini-RK technique was developed by Lindstrom[6] as an alternative incisional technique. The goal of this method is to reduce invasiveness, instability, hyperopic shift in the long term, and to maintain refractive effectiveness. The incision length is shortened by 50% to 60% in mini-RK. These short incisions may be expected to have slightly less effect, but can enhance corneal stability. Lindstrom[6] found a decrease in effectiveness of about 10%, but no postoperative hyperopic shift within 2 years after mini-RK. In our clinical study, however, we found approximately 23% less effectiveness in mini-RK eyes (unpublished data) compared to regular RK eyes at 1-year follow-up, which almost parallels the lower percentage of shallow incision (26.1%) found in the mini-RK incision group.

Our experimental histological study proved that the intended depth rate was not achieved in mini-RK eyes. The mean achieved incision depth rate in mini-RK was 47.28% (range 40.0–55.0%) versus 73.47% (range 60.5–79.1%) in conventional RK eyes. Minimally invasive radial keratotomy incisions were 26.1% more shallow than the conventional RK incisions. Our explanation for the shallow incision in mini-RK is that making a shorter incision does not allow enough time and distance for the diamond blade to cut deeply enough because cutting starts from the midpheripheric cornea. The effect of an incision is directly related to its length and depth; longer corneal incisions create more refractive effect.

In conclusion, it was demonstrated that one of the reasons for less refractive effect in mini-RK eyes was the shallow incisional depth. On the other hand, less epithelial plug formation was an advantage of this technique in terms of preventing long-term hyperopic shift and traumatic corneal rupture. Much less and limited histologic changes in the corneal structural integrity could also be viewed as proving the noninvasiveness of this technique. Finally, this study shows the short-term comparison results of these two incision techniques. Our long-term study on this subject is ongoing.

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