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Histologic and Immunohistochemical Evaluation of Radiosurgery in Strabismus Surgery in a Rabbit Model

Demet Yolcu[a], Ahmet M. Hondur[b], Nalan Akyürek[c], and Kamil Bilgihan[b]

[a]Department of Ophthalmology, University of Health Sciences, Abdurrahman Yurtaslan Oncology Training and Research Hospital, Ankara, Turkey; [b]Department of Ophthalmology, Gazi University Medical School, Ankara, Turkey; [c]Department of Pathology, Gazi University Medical School, Ankara, Turkey

ABSTRACT

Purpose: The aim of this study was to evaluate the use of monopolar radiosurgery (MRS) assisted strabismus surgery and to compare its histologic and immunohistochemical wound healing outcomes with conventional surgery.

Methods: Superior rectus muscle resection was performed on 30 white rabbits with three different surgical muscle cutting techniques: monopolar radiosurgery (MRS group), conventional scissors preceded by bipolar electrocautery (BEC group), and conventional scissors with no cauterization (control group). Degree of tissue injury, bleeding, inflammation, and fibrosis, as well as wound healing rate (CD68+ cell number), were evaluated.

Results: In CS group, hemorrhage scores were significantly higher than those in the other groups (MRS group: $Z = 5.182$; $p < 0.001$ and BEC group: $Z = 4.463$; $p < 0.001$) and MRS group had lower scores than BEC group; however, the difference was not significant ($Z = 1.423$; $p = 0.211$). The tissue injury score in BEC group was higher when compared with MRS, and the difference was statistically significant ($p = 0.028$). Median inflammation scores at days 1 and 21 were lowest in MRS group, but the difference was not statistically significant among groups (day 1; $p = 0.115$, day 21; $p = 0.095$). The median fibrosis score was higher in the control group, when compared with MRS, and the difference was statistically significant (muscle–sclera; $p = 0.011$ and muscle–conjunctiva: $p = 0.003$). The macrophage score (number of CD68+ cells) was lowest in CS group; however, the difference was not significant ($p = 0.657$).

Conclusions: Monopolar radiosurgery is a novel method for strabismus surgery and provides equivalent hemostasis effects and wound healing properties, compared with conventional methods, and enhances surgeon comfort, as muscle incisions are made in one step with clean surgical area.

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Monopolar radiosurgery; bipolar electrocautery; wound healing; extraocular muscle surgery; strabismus

Introduction

The formation of fibrous scar tissue is one of the serious complications following strabismus surgery and may originate from excessive bleeding, cauterization, and improper tissue handling.[1] Managing fibrous scar tissue around the extraocular muscle is often difficult, and several types of barrier materials, amniotic membrane grafting, antimetabolites, and anti-fibrotic drugs have been used to prevent the formation of adhesions.[2–14] Additionally, minimally invasive strabismus surgery techniques have been developed in an effort to reduce tissue traumatism and fibrous scar formation.[15,16]

Traditionally, extraocular muscle incisions are made by conventional Westcott scissors. The primary disadvantage of this method is the bleeding that obscures the surgical field and which usually calls for hemostasis through bipolar electrocautery (BEC). In the literature, various methods have been introduced to facilitate tissue incisions with minimal bleeding. Monopolar electrosurgery (MES) is the leader among these methods and has been clinically available for more than a half century; today it is in widespread use as an alternative to scissors.[17–20] Monopolar radiosurgery (MRS) is an electrosurgery instrument developed to reduce damage to surrounding tissue which prevents bleeding while making incisions, and its efficacy and reliability have been established in various ophthalmic operations.[21–31]

In our study, we used MRS for extraocular muscle surgery in a rabbit model and evaluated its clinical and wound-healing properties by comparing it with conventional methods. To the best of our knowledge, this is the first experimental study attempting a histological and immunohistochemical comparison of wound-healing properties after extraocular muscle surgery performed by MRS.

Materials and methods

We performed a prospective and experimental study on 60 eyes of 30 New Zealand albino rabbits weighing 2.5–3 kg each. The study protocol was approved by the Gazi

CONTACT Demet Yolcu drylcdemet@hotmail.com Department of Ophthalmology, University of Health Sciences, Abdurrahman Yurtaslan Oncology Training and Research Hospital, 06200 Demetevler, Vatan Cd, Ankara, Yenimahalle, Turkey.

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University Institutional Animal Care and Use Committee, in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgical methods

Eyes were randomized into the three groups, and three different surgical techniques were performed. Anesthesia, consisting of 30 mg/kg of ketamine hydrochloride (Ketalar, Eczacı başı , İstanbul, Turkey) and 3.5 mg/kg of xylazine hydrochloride (Rompun, Bayer, İstanbul, Turkey), was administered by intramuscular injection. All surgeries were performed under an operating microscope by one surgeon who was experienced with the ablative devices.

Surgical antisepsis with polyvinyl povidone–iodine (Betadine) was applied to the eyelids. Next, the superior rectus (SR) muscle was isolated on a muscle hook, placed on a Wright Grooved Muscle Hook, and sutured by a double-armed 6-0 polyglactin 910 Vicryl suture (2 mm) behind the resection point, and then a 4 mm SR muscle resection was performed. In the MRS group, after suturing the muscle, resection was performed by using an insulated needle electrode (vari-tip Ellman TNA8B) and MRS device (Ellman International, Hewlett, NY, USA) in cut–coagulate mode at power 1.5 (Figure 1(A,E)). In BEC group, hemostasis was performed to the vessels overlying the muscle using a BEC device (EK-160 Üzümcü) and bipolar electrocautery forceps at power 2, before suturing and cutting the muscle by scissors (conventional strabismus surgery method) (Figure 1(B)). In the CS group, cutting of the muscle was performed by Westcott scissors without using an ablative instrument for hemostasis (Figure 1(C)). The SR muscle was sutured to its original insertion point after resection, and the edges of the conjunctival peritomy were closed with interrupted 7-0 polyglactin 910 sutures (Vicryl) (Figure 1(E)).

A vasoconstrictor was not used at the operation site, and 3 mg/ml lomefloxacin eye drops (Okacin; Novartis, Pharma) were applied postoperatively. Half of the rabbits in each group were sacrificed at day 1 or 21 after surgery, and eyes were enucleated bilaterally, preserving the intact surgical field. Samples were fixed in 10% neutral-buffered formalin solution for histopathological examination and immunohistochemical staining.

Clinical evaluation

The amount of peroperative hemorrhage and tissue charring was evaluated at the time of surgery by using subjective grading scales, and each eye was rated individually (Table 1). Peroperative bleeding was evaluated by the number of triangular cellulose eye sponges that were used during surgery.

Histologic and immunohistochemical analysis

The study was randomized, and specimens were evaluated by one pathologist blinded to the characteristics and methods of the study. Each sample was processed for histologic examination by staining with Masson's trachoma (MT) and hematoxylin-eosin (H&E). To evaluate the severity of postoperative inflammatory infiltration and the extent of fibrosis, samples were examined using a light microscope (Olympus B × 51, Tokyo, Japan), and three representative high-power fields (HPFs) were evaluated as a proportion of the overall surgical wound cellularity or amount of fibrosis and scored according to the subjective grading scale (Table 1).

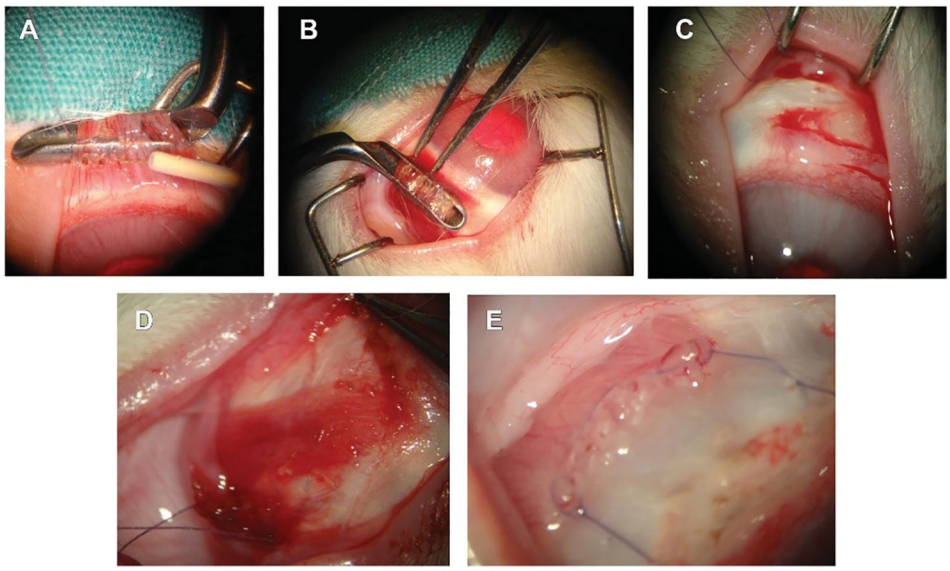


Figure 1. **A:** Representative picture of the SR muscle resection performed by MRS and insulated needle electrode. **B:** Representative picture of hemostasis performed by BEC and grade 2 tissue discoloration. **C:** Representative picture of muscle cutting performed with scissors and leakage hemorrhage. **D**: Representative picture of muscle cutting performed by BEC and Westcott scissors and grade 2 tissue discoloration. **E:** Representative picture of muscle cutting performed by MRS and grade 0 discoloration. The microhemostasis effect is visible within the tissue layers.

Table 1. Grading scales to determine instrument performance for bleeding, discoloration, and wound healing properties.

	Hemorrhage	Tissue effect	Inflammation	Fibrosis	CD68 (PGM1) cell count
Score 0	None	None	None	None	None
Score 1	1 sponge used	Slight discoloration	10% cell density	Mild fibrotic reaction around muscle	4 cells
Score 2	2 sponges used	Mild brown discoloration	10%–30% cell density	Easily detected thick bands	4–12 cells
Score 3	3 sponges used	Moderate brown discoloration	30%–50% cell density	Well-developed, dense bands of collagen	12 or more cells
Score 4	4 sponges used	Tissue charring	Above 50% cell density	Severe fibrotic response replacing large areas	–

To evaluate the wound healing rate, 21-day specimens were stained with CD68-PGM1, as described below, and the amount of macrophage and histiocyte cell density was determined and scored according to the subjective grading scale (Table 1). Formalin-fixed, paraffin-embedded tissue sections were cut to 3 µm, and an UltraView (Roche, USA) Universal DAB Detection Kit compatible with the device was used for IHC staining. Monoclonal mouse primary antibody CD68 (1:200 dilution, citrate cat. no: Ma5-12407 clone: pgm; Thermo Fisher Scientific CA, USA) was applied, and staining was completed according to the standard procedures. Tonsil tissue was used as a positive control for CD68 (PGM1) immunostaining.

Descriptive statistics of the data were presented as median (interquartile range; IQR) values. To determine differences between the groups, we used the Kruskal–Wallis nonparametric variance analysis or Mann–Whitney and Bonferroni-corrected Mann–Whitney tests. Variables among the groups were compared using the Spearman rank correlation coefficient test. We used SPSS for Windows v. 15 software (SPSS Inc., Chicago, IL, USA) for statistical analyses. A p value ≤ 0.05 was accepted as statistically significant for all tests.

Results

Among the three groups, peroperative bleeding was evaluated by the number of triangular cellulose eye sponge that were used during muscle resection. Some level of hemostasis was achieved in the BEC and MRS groups; however, in the CS group, additional effort and time were required for hemostasis, and hemorrhage scores were significantly higher than those in the other groups (MRS group: $Z = 5.182$; $p < 0.001$ and BEC group: $Z = 4.463$; $p < 0.001$). When we compared MRS and BEC groups, MRS had the least amount of bleeding; however, the difference was not significant ($Z = 1.423$; $p = 0.211$) (Figure 2).

We evaluated tissue injury by gross appearance of the muscle and determined the greatest amount of coagulation and brown discoloration in BEC group (Figure 1(B,D)), white blanching of the margins and limited tissue coagulation in MRS group (Figure 1(A,E)), and no evidence of thermal damage in the CS group (Figure 1(C)). We compared tissue injury between the BEC and MRS groups, and scores were higher in BEC, and the difference was statistically significant ($\chi^2 = 4.880$; $p = 0.028$).

When we evaluated early inflammation scores at day 1, MRS group had the lowest scores and BEC group the highest; however, there was no significant difference between the groups ($\chi^2 = 4.325$; $p = 0.115$). Similarly, we evaluated late inflammation scores at day 21, and MRS group had the lowest scores and BEC group the highest; however, there was no significant difference between the three groups ($\chi^2 = 4.720$; $p = 0.095$) (Figures 3 and 4(B)).

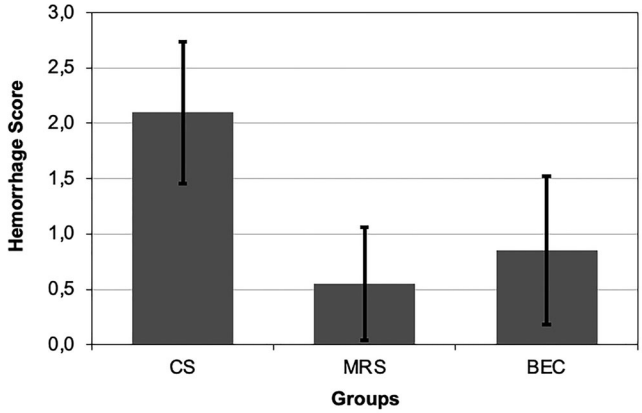


Figure 2. Graphical representation of median hemorrhage scores among the three groups (standard deviation bar).

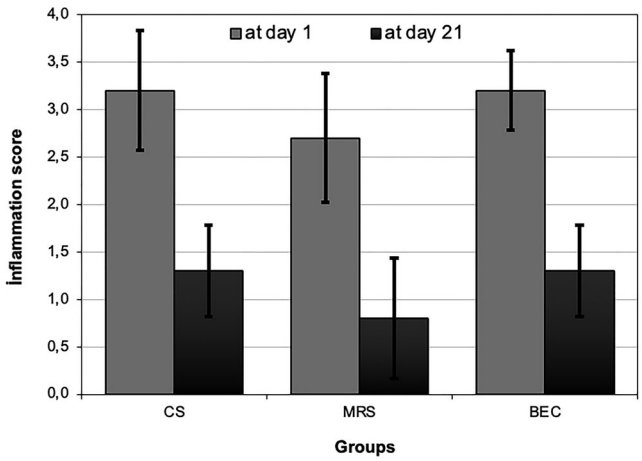


Figure 3. Representative graphic of median inflammation scores at days 1 and 21 among the three groups (standard deviation bar).

We evaluated the fibrosis scores, and muscle–sclera and muscle–conjunctiva fibrosis scores were highest in the CS group; when compared with MRS group, the difference was statistically significant (muscle–sclera: $Z = 2.541$; $p = 0.011$ and muscle–conjunctiva: $Z = 3.008$; $p = 0.003$) (Figures 4(A) and 5).

The wound healing rate was evaluated, and the macrophage scores were determined according to the CD68+ cell counts in day 21 samples (Figure 4(D)). The number of macrophage cells was lowest in CS group and highest in BEC group; however, differences between the three instruments were not statistically significant ($\chi^2 = 0.840$; $p = 0.657$).

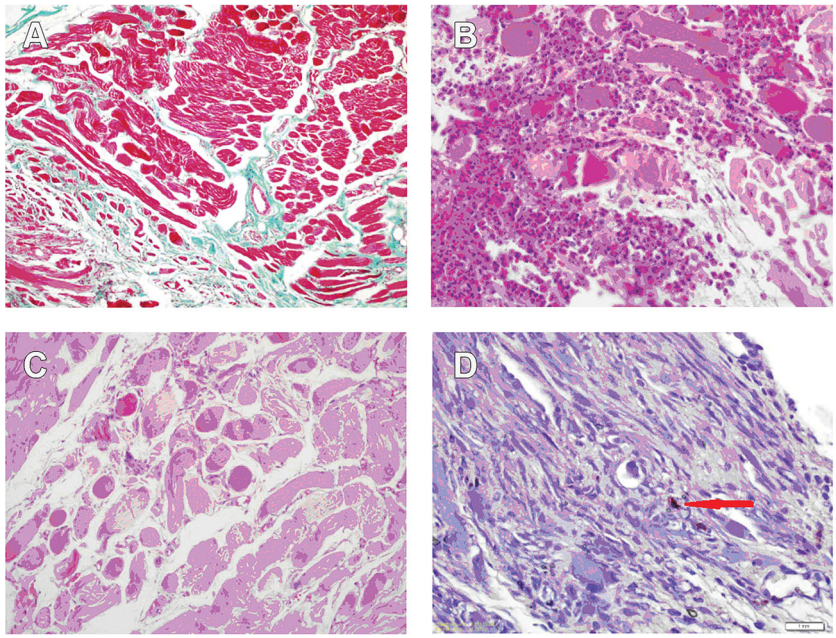


Figure 4. **A:** Grade 2 fibrosis score between the muscle–sclera and muscle–conjunctiva tissues at day 21, stained by MT. **B:** Grade 3 inflammation score at day 1, stained by H&E. **C:** Grade 1 inflammation at day 1, stained by H&E. **D:** Grade 1 macrophage (red arrow) density at day 21, determined by CD68 PGM1 immunohistochemical evaluation to establish wound healing rate.

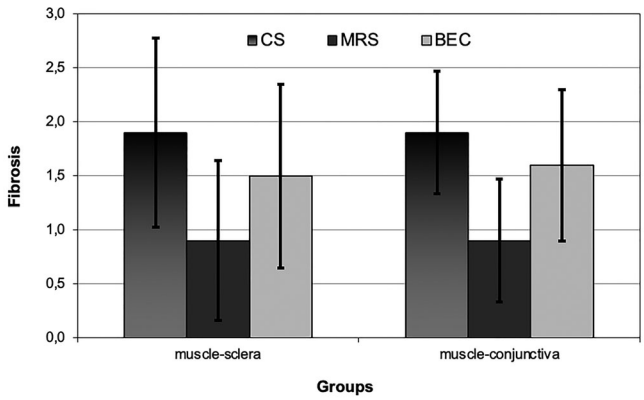


Figure 5. Representative graphic of the median muscle–sclera and muscle–conjunctiva fibrosis scores among the groups (standard error bar).

There was a statistically significant positive correlation between the peroperative hemorrhage and muscle–sclera fibrosis scores ($\rho = 0.455$; $p = 0.012$). Similarly, there was a positive correlation between the peroperative hemorrhage and muscle–conjunctiva fibrosis scores; however it not statistically significant ($\rho = 0.337$; $p = 0.069$). There was a positive correlation between the tissue charring and muscle–sclera and muscle–conjunctiva fibrosis scores; however, it was not statistically significant ($\rho = 0.258$; $p = 0.272$).

Discussion

Monopolar radiosurgery incisions are made by an electrosurgery instrument that uses high frequency radio wave energy and operates at a frequency of 3–4 MHz (older electrosurgery instruments operate at 1–2.9 MHz). Few studies have been conducted to compare MRS with other ablative modalities.[32–35] Studies in the literature comparing hemostasis and tissue charring in MES and MRS found that MES had a superior hemostasis performance with a broader charring effect, when compared with MRS; these results have been documented and/or contradicted by several other studies.[18–20] With ablative devices, the extent of thermal injury is determined by the electrode, application time, and wave length of the energy. Some studies state that surgeons who prefer one instrument over the other can feel confident in the knowledge that both instruments are equally safe and effective when used properly. For example, in one study MRS was used with a large-sized electrode for the management of an open angle glaucoma-stab incision, and broad subconjunctival scarring with a high incidence of ostium closure was documented.[29] In our study, we used MRS and BEC properly and with full attention to all parameters that are important in the use of ablative devices. We used MRS in the blended mode (50% cut and 50% coagulation), at an appropriate power (as determined by an investigator experienced with ablative devices), and with an insulated needle electrode, applied almost three strokes to each muscle, and before applying the second stroke, we waited 2 to 3 s for tissue cool to obtain hemostasis with minimal tissue injury. However, in BEC group we did not used MES and chose larger electrode sized BEC for only superficial tissue hemostasis and broader tissue charring effect found in the BEC group.[18,20,35] In the BEC group, we applied BEC for superficial tissue hemostasis, cut the muscle using scissors, and compressed the sponge until the bleeding stopped. In the MRS group, we applied MRS for tissue cutting and compressed the sponge until the bleeding stopped and we found that, we needed fewer sponges and less compression time in

MRS group compared with BEC group. Additionally, we can conclude that the superior hemostasis effect of MRS is due to the micro-hemostasis effect within the tissue layers that is made possible by the insulated needle-type electrode.

In the literature, extensive inflammatory infiltration was reported adjacent to MRS incisions, when compared with other ablative devices or scalpel incisions.[35] However, in numerous studies of the investigated instruments, there were no statistically significant differences in histological or immunohistochemical findings, and no differences in the extent, intensity, and/or character of the inflammatory cell infiltrate in various tissue types were observed (36). Similarly, in the current study, tissue inflammation was lowest in the MRS group and highest in the BEC group; however, there was no significant difference among the groups in the early and late postoperative periods.

The formation of fibrosis is an important issue after strabismus surgery, and there are many studies that address this complication with a minimal amount of success.[1–16] In one study, Nezhat et al.[33] evaluated the ablative methods and concluded that neither MRS nor ultrasonic dissection is superior in the prevention of adhesion formation in the late postoperative period when performed by a surgeon experienced in the use of ablative devices. In our study, all surgeries were performed by a surgeon experienced in using ablative instruments; however, in BEC group larger electrodes were used, in CS group surgeries were performed without hemostasis, and we evaluated fibrosis at the operative site, not only in the muscle layer but also in the conjunctiva and sclera. As a result, we conclude that bleeding in CS group and tissue charring in BEC group are the major risk factors for postoperative fibrosis formation in our study.

Wound healing can be investigated with various methods. One method is the determination of wound healing rate by CD68+ cell density. In the current study, the wound healing rate [number of CD68(+) stained cells] was lowest in CS group, but the difference was not significant. Similarly, Mannivannan et al.[19] reported that a scalpel caused more bleeding and less damage to adjacent tissue, when compared with ES, and resulted in more surgical site vascularizing and faster wound healing. In another study, epithelial recovery was found to be faster in the scalpel group, when compared to MES; however, several other studies have not found statistically significant differences in the progress of re-epithelization among these instruments.[17–20]

Stretched scar after strabismus surgery is well documented in the literature and may occur months to years after primary procedure. The wound healing process consist of three phases: the inflammatory phase, which occurs within the first 3 days after wounding; the fibroplasia phase, which occurs within the next 10–14 days, as collagen synthesis begins. The final phase, remodeling, begins at day 21 and lasts for several years. During this phase, the original collagen gel is replaced with with more stable collagen, and scar tissue formation occurs. In our study, we evaluated wound healing rates and tissue fibrosis at day 21; however, the occurrence of stretched scar could not be evaluated in our study because 21 days did not provide enough time to observe this change. We recommend further studies to investigate the properties of late-phase wound healing.[20,31,33]

To our knowledge, this is the first experimental study attempting a histological and immunohistochemical comparison of wound healing after extraocular muscle surgery performed by MRS, and we found that MRS-assisted strabismus surgery is considerably superior to the conventional method in terms of clinical parameters and wound healing properties. Practice, education, and training are the keys to deriving the best results from ablative devices and achieving micro-hemostasis with a good surgical field of visibility. In conclusion, MRS is a novel method for strabismus surgery and provides equivalent hemostasis effects and wound healing properties, requires less time and effort, and enhances surgeon comfort, compared with conventional methods, as muscle incisions are made in one step with a clean surgical area. As a result, monopolar radiosurgery can be used for routine extraocular muscle surgeries as an alternative to BEC.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Demet Yolcu http://orcid.org/0000-0002-3114-0116
Ahmet M. Hondur http://orcid.org/0000-0003-3955-6869
Nalan Akyürek http://orcid.org/0000-0002-2644-4609
Kamil Bilgihan http://orcid.org/0000-0002-7562-1173

Data availability statement

The data that support the findings of this study are available from the corresponding author, Yolcu D, upon reasonable request.

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