Evaluation the Safety of a New Intraocular Mitomycin C Application Technique in the Rabbit

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ABSTRACT

Purpose: To evaluate the safety of a new intraocular mitomycin c (MMC) application technique in the rabbits**.**

Materials and Methods: Sixteen male New Zealand rabbits were used. The rabbits were divided into 4 study groups. 2µg MMC in group-A, 5µg MMC in group-B, 10µg MMC in group-C and 20 µg MMC in group-D were applied with MMC sandwich technique into right eyes of rabbits. In MMC sandwich technique, ciliary body was protected with air, and posterior pole was protected with perfluorocarbon. The left eye was used as a control group. The rabbits had one-month followed up. Intraocular pressures were measured at first week and month 1. Scotopic and photopic a- and b-wave amplitudes and implicit times were compared between control and study eyes. Multiple small slices that included the ciliary body, macula and optic nerve head were examined for the evaluation of MMC toxicity

Results: Intraocular pressure did not differ between the control and study eyes. Scotopic and photopic a- and b-wave amplitudes and implicit times of group A, B, C and D eyes were similar to those of the control eyes. In all of the study groups, the histopathololgic morphologies of the ciliary body, retina and optic nerve were similar to those of control group. Furthermore, retinal layer thicknesses were similar between the MMC injected eyes and the control eyes.

Conclusions: Our newly defined MMC sandwich technique permitted the use of MMC up to 20 µg concentrations without causing toxicity in the vital intraocular structures.

Keywords: Intraocular drug application, Mitomycin C, Toxicity, Retinal detachment, Proliferative vitreoretinopathy.

INTRODUCTION

Proliferative vitreoretinopathy (PVR) is the primary cause of failure after retinal detachment (RD) surgery.[1] Oral or intravitreal pharmacologic agents are introduced to prevent PVR development.[2-6] However, these agents are effective only over short time periods, and intraocular use of non-toxic doses has no preventive effect on PVR development yet.[5,6]

Mitomycin C (MMC) is a long-acting antineoplastic agent that has been used in extraocular surgeries such as pterygium extraction and trabeculectomy.[7,8] In extraocular surgeries, a 100-200 µg/ml concentration of MMC is temporarily applied only to targeted parts, which has low rates of intraocular toxicity. However, MMC cannot be used inside the eye clinically like other antiproliferative agents, which are applied as intravitreal injections owing to potential side effects on retina and ciliary body.

An animal experiment has shown that intravitreal injection of 4µg MMC causes retinal toxicity in rabbits.[9]

In this study, a new surgical technique which was called "the MMC sandwich technique" was defined. MMC sandwich technique provided temporary drug application while preventing MMC contact with crucial posterior segment structures such as macula, optic nerve head and ciliary body. The aim of this study was to evaluate the safety (whether the technique can prevent the drug coming in contact with macula, optic nerve head and ciliary body and protect these structures from potential drug toxicity or not) of MMC sandwich technique while delivering different concentrations of MMC.

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MATERIALS AND METHODS

Animals

Sixteen New Zealand White rabbits (age 3 months, average weight 12 kilograms) were used. The study was approved by the animal research ethics board of the Gazi University Faculty of Medicine (Registration no:G.Ü.ET-16.034). The Principles of Laboratory Animal Care (NIH publication No. 8623, revised 1985) and the ARVO Statement for the Use of Animals in Research were followed. The rabbits were anesthetized with an intramuscular injection of 35 mg/kg ketamine and 5 mg/kg xylazine before every procedure (surgery, intraocular pressure (IOP) measurement, electroretinography and sacrifice). The rabbits were followed up for 1 month. All of the rabbits were sacrificed with an overdose of xylazine, and their eyes were enucluated at the end of month 1.

Study groups

The rabbits were divided into four study groups; each group consisted of four eyes. Prepared MMC concentrations were 2 µg/0.1 mL in group A, 5 µg/0.1 mL in group B, 10 µg/0.1 mL in group C and 20 µg/0.1 mL in group D (These concentratios were calculated after adding 0.05 mL brillant blue dye). The left eyes of four rabbits were used as control group. The brillant blue dye was added to make MMC solution visible during intraocular application.

MMC sandwich technique

Three-port vitrectomy was performed to 16 eyes of 16 rabbits. Some perfluorocarbon liquid (PFCL) was injected to cover the optic disc, macula and major vascular arcades after vitrectomy. Afterwards, a fuid-air exchange was performed while keeping the PFCL over posterior pole. Then, MMC solution was injected through one scleral port between the PFCL and air. The MMC was removed from the eye with a fluid needle after one minute (Fig 1). Then, PFCL and air was changed with fluid

Monitoring Toxiticiy

a- IOP

Intraocular pressure measurements were performed at week 1 and at the end of month 1. IOP was measured using Tono-Pen AVIA.

b- Electroretinography (ERG)

Electroretinography (ERG; Vision Monitor, Mon2014D, Metrovision, France) was performed at month 1. The standart protocol of International Society for Clinical Electrophysiology of Vision was followed for the ERG measurements [10]. The dark-adapted scotopic and light-adapted photopic responses were recorded in group A, B, C, D and the control eyes and compared. The amplitudes and implicit times of a- and b-waves were calculated according to the output of automatic software.

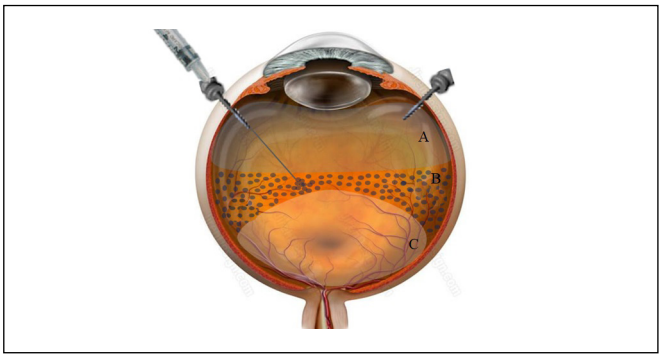

Figure 1: *Illustrution of mitomycin c sandwich technique:* ***A-****Partial air preventing MMC contact with anterior structures in order to avoid from MMC toxicity.* ***B-****Fluid with MMC overlying the targeted retinal areas.* ***C-*** *Perfluorocarbon liquid preventing MMC touch with posterior segment structures (macula and optic disc) in order to avoid from MMC toxicity.*

c- Histopathology

All of the eyes were enucluated at the end of month 1. The eyes were fixed for 1 week in 4% formaldehyde solution for histopathologic examination. The globe was cut away 3 mm behind the limbus after fixation procedure. Multiple small slices that included only the ciliary body, macula, optic nerve head ***(the sites where MMC contact was avoided)*** were obtained and stained with hematoxylin and eosin (H&E) for light microscopy examination. The sections were blindly examined with an Olympus DP22 light microscope (Tokyo, Japan) by a pathologist. Retinal layer thicknesses were measured seperately using morphometric analysis and compared between groups A, B, C, D and the control eyes. Morphometric analysis was performed with cellSens software (Olympus).

Statistical analysis was performed using SPSS version 22 (SPSS, Chicago, IL). Kruskal-Wallis test was used to compare continuous data between control and study groups. A p value less than 0.05 was considered to indicate statistical significance.

RESULTS

IOP

The IOP measurements of all of the groups at baseline, week 1 and month 1 were similar (Table 1).

ERG

There were no significant differences between MMC-injected eyes and control eyes in terms of a- and b-wave

Table 1: *Comparison of intraocular pressures between groups at week 1 and month 1.*

	Baseline Mean±SD	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	Group D Mean±SD	p
Week 1 IOP (mmHg)	14.6±1	14.6±1.4	14.9±0.6	15.2±1.3	14.6±1	0.904
Month 1 IOP (mmHg)	14.6±1	14.3±1.1	14.3±0.6	14.6±1	14.9±1.2	0.895

IOP: Intraocular pressure **Group A:** 2µg Mitomycin C **Group B:** 5µg Mitomycin C **Group C:** 10µg Mitomycin C **Group D:** 20µg Mitomycin C

amplitudes and implicit times under both scotopic and photopic conditions (Table 2).

Histopathology

The retina, optic disc and ciliary body had similar morphologic appearences with control eyes in all MMC-injected eyes in the light microscopic examination (Fig 2a-b, Fig 3a-b and Fig 4 a-b). There were no photoreceptor disarrangement in the retinal histopathologic examinations. In the morphometric analysis, MMC-injected eyes had similar retinal layer morphology and total retinal thickness measurements with the control eyes (Table 3).

DISCUSSION

Proliferative vitreoretinopathy is the primary cause of surgical failure of RD surgery.[1] Adjuvant medical treatments

Table 2: *Comprison of ERG parameters between mitomycin c injected and control eyes.*

Amplitude (mV)	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	Group D Mean±SD	Control Mean±SD	P
Scotopic a- wave	-11.2±1	-11.6±	-11.4±	-11.2±	-10.7±1	0.708
Scotopic b-wave	12±2	12.3±1.7	12.1±1.7	12.2±1.8	12.4±2.5	0.995
Photopic a-wave	-2.1±0.4	-2.07±0.9	-2.15±0.5	-2.15±0.4	-1.95±0.6	0.960
Photopic b-wave	11.6±1.1	11.8±1.2	11.7±1.2	11.6±1.1	11.9±1.2	0.931
Implicit time (ms)						
Scotopic a- wave	22.4±2.3	22.6±2.4	22.5±2.5	22.7±2.5	22.2±2	0.995
Scotopic b-wave	10.8±2.7	10.6±1.9	10.7±2.3	10.5±2	10.2±2.5	0.976
Photopic a-wave	22.6±0.3	22.3±0.6	22.3±0.6	22.4±0.4	22.3±0.4	0.851
Photopic b-wave	6.97±0.5	6.9±0.6	6.92±0.5	6.9±0.8	6.8±0.6	0.972

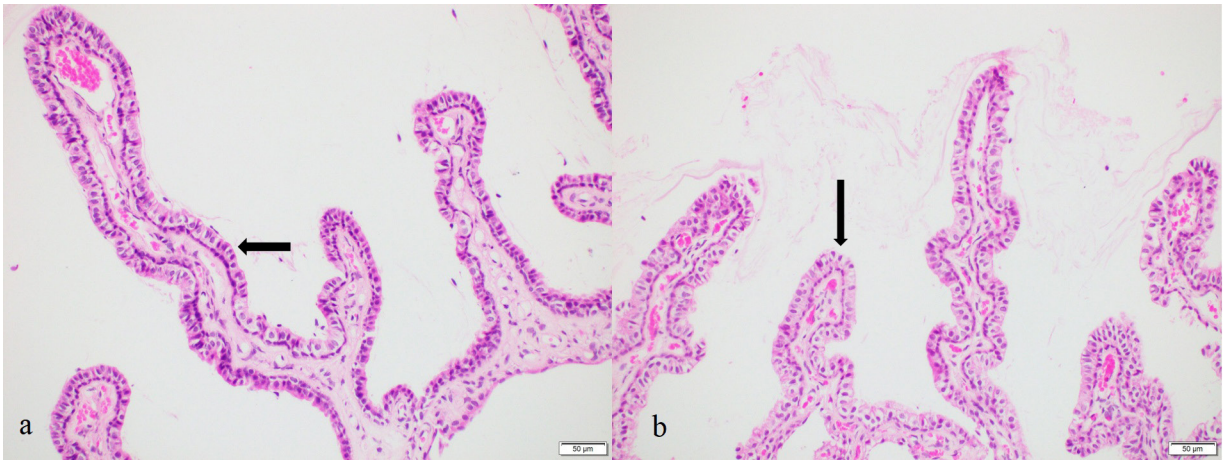

Figure 2: *a) Histopathologic morphology of ciliary body (H&E ×200) in mitomycin c sandwich technique 20 µg MMC subgroup. (Black arrow: Ciliary epithelium). b) Histopathologic morphology of ciliary body (H&E ×200) in control group (Black arrow: Ciliary epithelium).*

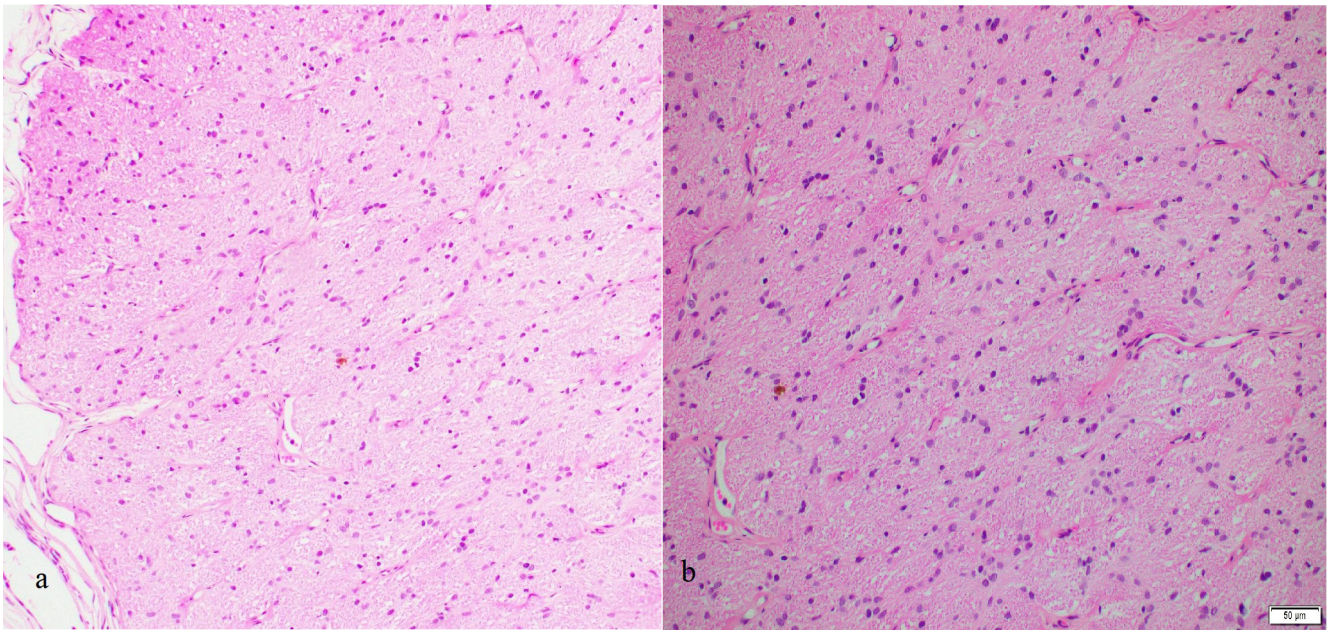

Figure 3: *a) Histopathologic morphology of optic disc (H&E ×100) in mitomycin c sandwich technique 20 µg MMC subgroup. b) Histopathologic morphology of optic disc (H&E ×200) in control group.*

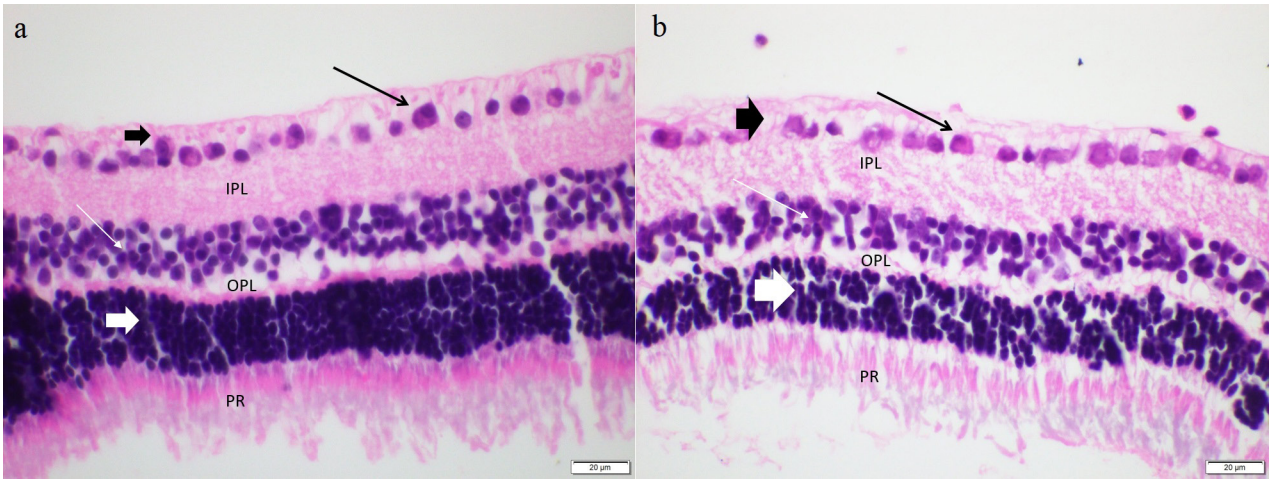

Figure 4: *MMC injected eyes had similar morphologic appearence with control eyes even at high concentrations a) Histopathologic morphology retina (H&E ×600) in mitomycin c sandwich technique 20 µg MMC subgroup (**Black thin arrow:** Ganglion cell, **Black thick arrow:** Ganglion cell layer, **White thin arrow:** Inner nuclear layer, **White thick arrow:** Outer nuclear layer, **IPL:** Inner plexiform layer, **OPL:** Outer plexiform layer, **PR:** Photoreceptor layer). b) Histopathologic morphology and morphometric measurements of retina (H&E ×600) in control group (**Black thin arrow:** Ganglion cell, **Black thick arrow:** Ganglion cell layer, **White thin arrow:** Inner nuclear layer, **White thick arrow:** Outer nuclear layer, **IPL:** Inner plexiform layer, **OPL:** Outer plexiform layer, **PR:** Photoreceptor layer).*

were used to improve success rates in the treatment or prevention of PVR. Some adjuvant treatments—including oral retinoic acid, oral and intravitreal steroids, 5-fluorouracil (FU) and low-molecular weight heparin and intravitreal daunorubicin—have been used clinically to prevent PVR development.[2-6,11] Additionally, many other agents have been used experimentally.[12-16] However, none the agents noted above have been used in routine clinical practice owing to the absence of a definite preventive effect on PVR development.

Mitomycin C is a long-acting antineoplastic agent that has been used in extraocular surgeries fors a long time.[7,8] Retinal and ciliary body toxicity can be observed, even after extraocular surgeries, due to drug penetration into the posterior segment, which makes it difficult to use MMC in intraocular surgeries.[17,18]

Table 3: *Comprison of retinal layer thicknesses between mitomycin c injected and control eyes.*

Thickness (µm)	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	Group D Mean±SD	Control Mean±SD	P
RGC	22±5.6	22.5±3.1	23±4	22.5±3.5	22,5±3.5	0.997
IPL	22.5±2.1	21±1.4	22.5±2.1	22.5±3	20±1.4	0.667
INL	20±1.4	19.5±2.1	19.5±2.1	19.5±2.5	19.5±2.1	0.996
OPL	12.5±2.1	13.5±2.1	13.5±1	13±1.4	14±2.8	0.922
ONL	30±4.2	30±5.6	33.5±3.5	30.5±4.9	32±6.4	0.930
PR	40.5±3.5	37.5±2.1	40±4.2	39.5±4.9	41±5	0.842
Total	147.5±10.6	144±11.3	152±9.8	147.5±12	149±9.8	0.745

RGC: Retinal ganglion cell **IPL:** Inner plexiform layer **INL:** Inner nuclear layer **OPL:** Outer plexiform layer **ONL:** Outer nuclear layer

In a previous experimental rabbit study, 0.5,1,2,4,8 and 16 µg MMC were injected into the normal vitreous; only 2µg of MMC was shown to be non-toxic to rabbit retinas according to the ERG responses.[9] Focal tractional changes (stage 2 and 3) occured with 1 µg MMC in 36.4% eyes in gas-compressed eyes. This finding suggests that higher MMC concentrations may prevent PVR development. However, in gas-compressed eyes, retina pigment epithelial cells have exhibited abnormalities such as decreased basal infoldings and increased cytoplasmic vacuoles in addition to considerable decreases in the b-wave amplitude with 2 µg of MMC. In clinical practice, vitreous gel is removed and an endotamponade (silicone or gas) is used in RD surgeries. Therefore, an intravitreal injection of 2 µg or less of MMC - as in Hu and Chung's study[9] - may not be applicable: first, in operated eyes, there is no vitreous gel as a reservoir for the drug. Additionally, there exists an endotamponade instead of normal vitreous, which means 2 µg of MMC may be toxic according to Hu and Chung. Furthermore, MMC may be in contact with the ciliary epithelium and lens and other vital structures around the endotamponade. In addition, 2 µg MMC may not remain in the operated eyes owing to possibility of increased clearence of MMC due to absence of vitreous gel; this situation may result in lower MMC concentrations in the eye. Lower concentrations due to increased clearance may not be able to prevent PVR development in more than one-third of cases according to Hu and Chung. Furthermore, a 1 or 2 µg application of MMC may cause ciliary body toxicity when the drug is left in the vitreous gel. Because, in Hu and Chung's study, the authors did not note IOP levels or histopathology of the ciliary body.

Mitomycin C is a potent antiproliferative agent. Intravitreal use via direct intravitreal injection and leaving the drug in eye may be toxic even with very low doses. Therefore, we attempted to use MMC temporarily in a situation similar to extraocular applications, which is called MMC sandwich technique. In extraocular applications, the MMC concentration is 200µg and the application time is 2 minutes. As a result, tissues are exposed to higher concentrations for longer durations than in our application technique. However, adverse effects, such as ciliary body and retinal toxicity, are reported with low rates owing to drug penetration into posterior segment and contact with these structures.[17,18] The originality of our application technique is prevention of MMC contact with vital intraocular structures and removal of the MMC. The MMC was prevented from coming in contact with the posterior pole structures, including major retinal arcades, macula and the optic disc, by covering these structures with PFCL. Mitomycin C contact with the ciliary epithelium and other anterior segment structures was prevented by filling the rest of the eye with air. When injected, a constant amount and concentration of MMC was only in contact with the intended areas of retina such as equatorial and midperipheral retina. After 1 minute all of the MMC was removed from the eye. Indeed, we did not encounter ciliary body, macula or optic nerve toxicity linked to our drug application technique. The functions of the ciliary epitheliums of MMC-injected eyes appeared to be unaffected because the IOP measurements were similar to baseline levels at week 1 and month 1. Additionally, the ciliary epitheliums had similar morphology as the ciliary epitheliums of control eyes (Figure 2a-b). We evaluated ERG measurements at month 1 owing to possible effect of vitrectomy on ERG responses. Because, previous animal studies have shown that vitrectomy transiently effected retinal function in the early postoperative period.[19,20] The ERG analysis did not reveal photoreceptor, bipolar cell or Müller cell functional disturbance; the a- and b-wave amplitudes and implicit times of MMC-injected eyes were similar to those of the control eyes at month 1. The retinal areas which was exposed to MMC may be expected to effect ERG measurements. However, those exposed areas were very small and may not have altered the ERG measurements. Light microscopic examinations demonstrated normal inner and outer retinal morphology

(Figure 4a-b). Morphometric analysis from the protected retinal areas revealed that retinal ganglion cell, inner plexiform, inner nuclear, outer plexiform, outer retinal and photoreceptor layer and total retinal thicknesses of MMC-injected eyes were similar to those of the control eyes. We did not detect any disarrangement at the photoreceptor layers. Briefly, the IOP measurements, ERG analysis and histopathologic examinations suggest that our newly defined application technique may prevent the drug from coming in contact with vital structures and thereby avoid toxic effects, even when doses exceed known toxic levels.

The aims of this study were to evaluate: first, whether MMC sandwich technique was applicable and second, whether various concentrations of MMC using this technique (even up to 20 µg) caused optic nerve head, macula, ciliary body and anterior segment structure toxicity or not than measuring the effectiveness of the drug. Our results suggest that MMC sandwich technique appears to be applicable and this technique may be tested to prevent postoperative PVR development in future experimental or clinical studies. This method also provides the opportunity to apply medication to a targeted area of the retina. Furthermore, this technique may provide a way to test various other drugs in both experimental or clinical PVR studies. MMC is toxic to intraocular tissues, even with low concentrations (2 µg in gas compressed eyes) according to Hu and Chung.[9] The outcomes of MMC sandwich technique suggest that higher MMC concentrations, even up to 20 µg, may be used safely without causing toxic effects on vital intraocular structures owing to protection these structures with PFCL and air. However, further studies are required to test long term outcomes or potential late toxic effects of temporary intraocular MMC application using this sandwich technique.

We did not evaluated the toxic effects of MMC on those small retinal areas where the drug was applied. In addition, current study was not designed to evaluate the therapeutic effects of intraocular MMC application on PVR development. Because, the aim of this study was to evaluate whether MMC came in contact with those protected areas and caused toxicity or not. We believe that future studies are required to determine the effect of various doses of MMC using this sandwich technique for the prevention of postoperative PVR development.

CONCLUSIONS

Our newly defined MMC sandwich technique appear to allow MMC application to targeted retinal parts and prevent the drug coming in contact with vital structures. Toxic effects of MMC can be avoided by this protective and temporary drug application technique. Higher doses up to 20µg of MMC may be used with this technique. Further animal studies are required to evaluate the effects of higher MMC concentrations with longer exposure duration (greater than 20 µg and longer than 1 minute) using this technique on PVR development.

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