The Effect of Mitomycin C on Corneal Endothelial Cells in Trabeculectomy

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ABSTRACT Objective: To investigate the effect of mitomycin C on corneal endothelial cells in trabeculectomy. Methods: 60 patients (78 eyes) were diagnosed with glaucoma and operated trabeculectomy in our hospital from September 2010 to May 2011, they were collected and randomly divided into group A and group B. All cases had undergone test for intraocular pressure (IOP), corneal endothelial cell density (CD) , average cell area(AVG) and cell area of variation(CV) before 1 month and 3 months after the operation, to analyze the changes of the number and the differences between the two groups. Results: The intraocular pressure (IOP) before the operation were (35.4± 13.7) mmHg in group A, and were (32.5± 13.5) mmHg in group B. There was no significant difference between the two groups (P＞0.05). The intraocular pressure (IOP) in the group A was (15.7± 3.7) mmHg and (17.0± 3.2) mmHg at 1 month and 3 months after the operation. The IOP was lower than the (19.4± 3.7) mmHg and (20.2 ± 2.1) mmHg in the group B. There was significant difference between two groups(P＜0.05). The corneal endothelial cell density, average cell area, cell area of variation before and 1 month and 3 months after the operation were (2475± 484) / mm^2, (2199± 373) / mm^2, (2164± 332) / mm^2; (431.4± 67.6) μm^2, (480.6 ± 66.8) μm^2 ,(463.8± 46.2) μm^2, (31.1± 7.4)%, (34.4± 6.3) %, (31.2± 7.5) %, respectively, in group A; The results for the group B respectively were (2342± 94) / mm^2, (2185± 215) / mm^2, (2074± 218) / mm^2; (453.9± 94.8)m^2, (516．3± 100.8)m^2, (499.81± 106.4) m^2; (30.2± 3.0) %, (32.7± 2.9) %, (31.4± 4.3) %. The first month and postoperative 3 months parameters and the comparison, are statistical. There was significant difference before the operation and 1 month or 3 months after the operation when endothelial cell density, average cell area, cell area of variation in group A were compared (P < 0.05). But there was no significant difference in group B (P＞0.05) except of the corneal endothelium after 3 months. The loss rate of corneal endothelium cells in the group A was 10.4% at 1 month after the operation, which was higher than the 6.1% in group B (P＜ 0.05). The loss rate of corneal endothelium cells in the group A was 11.1％at 3 months after the operation, which was higher than 10.0% that in group B (P＞0.05). Conclusion: The step-down effect of using mitomycin C is better than without mitomycin C in trabeculectomy，but the loss rate of corneal endothelial of the former is higher than the latter in short-term.

Key words: Corneal endothelium; Mitomycin(MMC); Trabeculectomy

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Introduction

Functional bleb formation of trabeculectomy is the ideal goal. Antimetabolites mitomycin C can inhibit proliferation of cells, DNA replication and synthesis, fibroblast proliferation, prevent fibroblast cells to produce collagen, reduce scar formation in the mouth of filtration, enhance the success rate of surgery, but mitomycin C inhibites cell proliferation while the cells have a certain toxicity, and has a dose-and time-dependence, short-term exposure to high concentrations of MMC in addition to inhibition of fibroblast proliferation, there is a strong anti-cells [3]. This study investigated the changes of the effect of mitomycin C on corneal endothelial cells in trabeculectomy before the operation, 1 month after and 3 months after the operation.

1 Materials and Methods

1.1 Clinical data

60 patients (78 eyes) were collected from September 2010 to May 2011. They were diagnosed with glaucoma and trabeculectomy in line with indications in the Affiliated Hospital of Qingdao University Medical College. According to the use of mitomycin C or not, they were divided into Group A and Group B. Patients who use mitomycin C in group A, no mitomycin C in group B. There were 60 patients (78 eyes) in Group A, There were16 males cases (20 eyes), 20 females (26 eyes), they were aged from 27 to 77 years, average (57.5 ± 10.7) of age. Primary acute angle-closure glaucoma 12 patients (12 eyes), chronic angle-closure glaucoma 22 (30), developmental glaucoma 2 cases (4), onset time of 1 day to 3 years. There were 24 patients (32 eyes) in Group B, of which male 12 (16) and 12 females (16), aged from 41 to 80 years, average (57.8 ± 14.3)years, with chronic angle-closure glaucoma, 16 patients (24 eyes), acute angle-closure glaucoma in 8 cases (8), onset time of 1 day to 2 years, excluding ocular trauma and surgery.

1.2 Surgical methods

General anesthesia, cut along the top of the limbal conjunctiva, make 5 × 5 mm^2 scleral flap about 1 / 2 tongue-shaped scleral thickness, flap, cotton sheet placed 0.3mg/ml MMC 3min, fully

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saline flush, under anterior chamber paracentesis, removal of 1 × 2mm^2 corneoscleral trabecular tissue, and cut the appropriate parts of the peripheral iridectomy, 10-0 nylon interrupted suture the scleral flap 3-pin, 2 pin is removable scleral suture, before the formation of room, check the edge of the scleral flap was no obvious leakage, conjunctival sutures. Surgery eye drops 0.5% Cravit, tobradex and Putnam flutter Ling eye drops, 4 times a day, continue 3-4 weeks.

1.3 Outcome measures

1.3.1 Intraocular pressure The intraocular pressure was measured before and 1 month and 3 months after the operation by using non-contact tonometer IOP. According to the intraocular pressure, bleb, anterior chamber，adjusted line can be removed,If the bleb appeared mild fibrosis, according to each review, give fluorouracil, 5mg subconjunctival injection.

1.3.2 Examination of corneal endothelial cells Japan TOPCON SP-2000P corneal endothelial microscopy was used to exam corneal endothelial cells of undergoing eye respectively, before surgery, 1 month and 3 months after operation Select parts of a unified measurement of central corneal area, take 30 to 50 endothelial cells, measured by the computer analysis system endothelial cell parameters, record cell density, average cell area, coefficient of variation of cells, endothelial count to check twice to take the average.

1.4 Statistical Analysis

SPSS17.0 statistical software was used for data processing. Measurement data was presented as mean ± SD ($\bar{x}\pm s$) .The test of the significance of difference among the groups were using analysis of variance, two groups comparison were determined by using t-test, and the P <0.05 as statistically significant difference.

2 Results

2.1 A, B groups IOP changes before and after surgery

There was no significant difference in preoperative IOP differences between the two groups (t=1.054, P=0.295) . There was significant difference before the operation and 1 month or 3 months after the operation in two group (P <0.05). The intraocular pressure in group A was lower than that it in group B at 1 month and 3 months after the operation (t=-1.951, P=0.049; t=-3.430, P = 0.001) (Table 1).

Table 1 Intraocular pressure of the two groups before and after surgery

	Preoperative	1mon postoperation	3 mon postoperation
A	35.4± 13.7	15.7± 3.7*	17.0 ± 3.2*
B	32.5± 13.5	19.4± 3.7*	20.2 ± 2.1*

Note:*P＜0.05.

2.2 A, B groups before and after surgery, corneal endothelial cell changes

Preoperative A, B groups of corneal endothelial cell density, average cell area, coefficiency of variation of cell area difference was no statistically significant (P > 0.05)，There was significant difference before the operation and 1 month or 3 months after the operation when endothelial cell density, average cell area, cell area of variation in group A were compared (P < 0.05) . But there was no significant difference in group B (P > 0.05), except of the corneal endothelium after 3 months(Table 2).

Table 2 Corneal endothelium cell density and cellular morphology of the two groups before and after surgery (Mean± SD)

	Group A			Group B		
	AVG(um^2)	CV(%)	CD (mm)	AVG(um^2)	CV(%)	CD (mm)
Preoperation	431.4± 67.6	31.1± 7.4	2475± 484	453.9± 94.8	30.2± 3.0	2342± 94
Lmonpostoperation	480.6± 66.8*	34.4± 6.3*	2199± 373*	516.3± 100.8	32.7± 2.9	2285± 215
3monpostoperation	463.8± 46.2*	32.0± 6.7*	2164± 332*	499.81± 106.4	31.4± 4.3	2174± 218*

Note:*P＜0.05.

2.3 The loss rate of corneal endothelial in two groups postoperative

There were significant difference for 1 months postoperation endothelial cell loss rate in two groups (t = 0.036, P <0.05). But there was no significant difference for 3 months postoperation endothelial cell loss rate in two groups were compared(t = 0.531, P> 0.05)(Table3).

Table 3 The loss rate of corneal endothelial in two groups postoperative (Mean± SD)

	1mon postoperatio	3mon postoperatio
A	0.1034± 0.08 (10.34%) *	0.111± 0.135 (11.1%)
B	0.060± 0.043(6%)*	0.100± 0.094(10%)

Note:*P＜0.05.

3 Discussion

The aim of trabeculectomy was to build a filtration channel and make the successful drainage of aqueous humor by reducing the intraocular pressure. So preventing filtration wound healing and reducing scar formation is critical to improve achievement ratio of operation. Antimetabolites mitomycin C has the function of inhibiting fibroblast proliferation and reducing the filtration port scar formation. But as the cell cycle is relatively non-specific drugs, it can inhibit DNA synthesis [17]. Holger M proposed that people in the application of MMC trabeculectomy to improve surgical success rates at the same time should pay attention to that it may cause side effects [4]. Transparent cornea is one of the important factors to achieve the physiological function of visual organ, anatomic form Integrity and physiological functions of normal corneal epithelium is key to maintaining corneal transparency [1]. Reported in the literature: the corneal endothelial cell area and density is an important indicator to detect corneal endothelial cell function and functional reserves [2]. Inspection of the corneal endothelium to the corneal endothelial cells morphology, data processing and analysis, assessment of corneal function can help guide the diagnosis and assessment of certain eye diseases of the cornea against certain diseases.

Through the application of mitomycin C , clinician tries to improve the success rate of trabeculectomy, Pasquale useing monkeys found persistent high intraocular pressure model, filtration surgery 1 month after application of MMC and postoperative 2 months of intraocular pressure were lower than those in the application of balanced salt solution group, the difference was statistically significant [12] . Tang in 53 patients with refractory glaucoma underwent trabeculectomy found that IOP after MMC group than in the control group eyes down, the difference was statistically significant [13]. The study also found that application of MMC after 1 month and 3months after surgery without intraocular pressure lower than the MMC group, indicating that the trabeculectomy scleral flap placed MMC, inhibited the scleral fibroblast population of value-added filtration, the filtration duct patency to enhance the drainage of aqueous humor. Meanwhile, MMC has some of the ciliary process resulting in the inhibition of aqueous humor secretion [14]. Storr-PaulsenT operated 14 cases of open-angle glaucoma trabeculectomy with mitomycin C and observed that the rate of cell loss were 9.5% and 10% 3 months postoperation and 12 months postoperation when , it was similar to the study of 3 months postoperation [5] . GAO found that the corneal endothelial cell density decreased 9.5% after the trabeculectomy with MMC 3 months postoperation [6]. And after 3 months of the study 11.1% wasrelatively low endothelial loss , some studies show that [11] open-angle glaucoma and non-acute episode of angle-closure glaucoma and corneal endothelial cell density compared to normal was no significant difference, while a history of acute onset open-angle glaucoma, corneal endothelial cell density was significantly lower than normal, the cell area was larger, and the size obvious different.

Lá zaro GC operated 35 cases the ages of 65 to 75 open-angle glaucoma patients with trabeculectomy，after 3 months he observed the rate of corneal endothelial cell loss 6.35% [7]. But Barak found that the rate of corneal endothelial cell loss (16.6 ± 11.1)% [8]. The results were different from our experiment. It is remarkable that this group of patients in the postoperative follow-up applied fluorouracil, which may increase the loss of corneal endothelial cell. Studies showed that Toxicity of 5-FU on the corneal endothelium maybe lead to a series of changes in the intracellular environment [18] . Caused by changes in cell function, increase the permeability of cell membranes, leading to cell edema. The effects of 14 successive daily subconjunctival 5-fluorouracil (5-FU) injections on corneal endothelial and ciliary epithelial cells were studied in pigmented rabbits. Araie M, with scanning or transmission electron microscopy , found that the corneal endothelial cells showed moderate cytoplasmic swelling and mitochondrial swelling and vacuolation [19]. The changes were dose-dependent and most marked in the area near the injection site. Nuyts RM set up an in vitro system to establish a dose-response effect [20]. Cytotoxicity of MMC and 5-FU was quantified using Mosmann's colorimetric assay in a bovine endothelial cell culture system. And they found that Cytotoxicity of MMC and 5-FU was related to the dose and time of using. All in all, loss of corneal endothelial cells with the following different conditions may relate to: 1 .duration of preoperative high intraocular pressure2 with or without acute episode 3. The concentration and time of intraoperative mitomycin C 4 individual response to surgery and drugs differently. Studies [15,16] suggest that a long time high intraocular pressure and a significant short-term high IOP and shallow anterior chamber will cause the aqueous barrier, resulting in the anterior chamber metabolic toxic products accumulate, Nutrients capacity and concentration of the aqueous reduction in corneal endothelial hypoxia, decreased physiological function, and irreversible damage. The density decreased and size increased of corneal endothelial cell had relevance to the time of acute attack and high intraocular pressure. The longer the duration of attack the greater the damage.

Intraocular surgery on corneal endothelial cells had different degrees of damage, making the corneal endothelial cell density decreased. The repair of corneal endothelial cells relied on endothelial cell migration and expansion, so the average corneal endothelial cell area is increased. Joyce [9] and Amann [10] studies suggest that the regeneration characteristics of human corneal endothelial cells are related to the age, time, and damaged parts of the cornea, the younger, damaged shorter, more damaged parts of the endothelial cells near the periphery the mitotic stronger. Studies have found that trabeculectomy with MMC before and after corneal endothelial cell loss was observed 3-12 months, the cell loss did not

progress [6], MMC on the corneal endothelial cells without toxic side effects of sustained .

The study found that two groups of corneal endothelial cell damage mainly in the postoperative 1 month, each parameter change in the trend gradually decreases after 3 months, and the loss rate of corneal endothelial cell in group A is higher than that in group B after 1 month, but the two groups have no difference in endothelial cell loss rate after 3 months, which indicated that the loss of corneal endothelial cells gradually decreases,and the repair of corneal endothelium itself gradually strong.

References

[1] Fan J. Corneal Endothelial Cell Function and Clinical Significance[J]. Liuzhou Medical,2009,22(3):160-162

[2] Qu C, Sheng MJ, Lin AJ. Morphological Study on Endothelial Cells of Mid-term Stored Corneas in Rabbit [J]. Journal of tongji university (medical science),2004,25(5):374-376

[3] Zhang SX, Liu L. Glaucoma Therapy [M]. Beijing: People's Health Publishing House,1998:222-235

[4]] Mietz H, Roters S, Krieglstein GK. Bullous keratopathy as a complication of trabeculectomy with mitomycin C [J]. Graefe's Arch Clin Exp Ophthalmol,2005,243:1284-1287

[5] Norregaard JC, Ahmed S, Storr-Paulsen A. Corneal endothelial cell loss after mitomycin C-augmented trabeculectomy [J]. J Glaucoma, 2008,7(8):654-657

[6] Gao FJ. The Effect of Mitomycin C on Corneal Endothelial Cells in Trabeculectomy[J]. Chin J Pract Ophthalmol,2010,28(9):1040-1042

[7] Lá zaro GC, Castillo GA, Garcí a FJ, et al. Study of the corneal endothelium after glaucoma surgery [J]. Arch Soc Esp Oftalmol,2000,75 (2):75-80

[8] Barak A, Alhael A, Kotas R. The protective effect of early intraoperative injection of viscoelastic material in trabeculectomy [J]. Ophthalmic Surg,1992,23(3):206-209

[9] Joyce NC. Proliferative capacity of the corneal endothelium [J]. Prog Retin Eye Res,2003,22(3):359-389

[10] Amann J, Holley GP, Lee SB, et al. Increased endotlIelial cell density in the paracentral and pefipheral regions of the human cornea[J]. Am J Ophthalmol 2003,135(5):584-590

[11] Luo Y, LI JZ, Jin YM. Changes of Endothelium in Patients with Primary Glaucoma[J]. Acta academiae medicinae sinicae,2000,22(4) : 356-359

[12] Pasquale LR, Thibault D, Dorman-Pease ME, et al. Effect of topical mltomycinc on glaucoma filtration surgery in monkeys [J]. Ophthalmology,1992,99(1):14-18

[13] Tang X, Zhang SX, Liu L. Mitomycin C in refractory glaucoma cord surgery[J]. Ophthalmology,1995,4(1):32-35

[14] Xia XB, Jiang YQ, Huang PG, et al. Mitomycin C on ciliary body of rabbit eyes without pigment epithelial cell toxicity[J]. Chinese Journal of Ophthalmology,1998,34:190-193

[15] Chen XY, QI Y, LU XR, et al. Effect of primary glaucoma on the corneal endothelium [J]. Recent advances in ophthalmology,2006,26 (4):294-295

[16] Liu JR, Ma QL. Effect of primary glaucoma on the corneal endothellal cell of clinical research [J]. Journal of clinical ophthalmology, 2007,15(5):394-395

[17] Wang WM, Yang B, Ma ZZ. The application of mitomycin C in ophthalmology[J]. Qianwei journal of medicine & pharmacy,2001,18 (1):71-72

[18] Sun YX, Li XR, Zhang XH, et al. Study On inhibition of rabbit lens epithelial cells proliferation and intraocular toxicity by 5-Fu[J]. Ophthalmology,2000,18(3):224-226

[19] Araie M, Nakano Y, Akahoshi T. Effects of subconjunctival 5-fluorouracil injections on the corneal endothelium and ciliary epithelium [J]. Graefes Arch Clin Exp Ophthalmol,1990,228(6):573-581

[20] Nuyts RM, Pels E, Greve EL. The effects of 5-fluorour acil and mitomycin C on the corneal endothelium [J]. Curr Eye Res,1992,11(6):565-570

小梁切除术中应用丝裂霉素 C 对角膜内皮细胞的影响

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摘要 目的:观察小梁切除术中应用丝裂霉素 C (MMC) 对角膜内皮细胞的影响。**方法**:收集 2010 年 9 月 2011 年 5 月在我院行小梁切除术的青光眼患者 60 例 (78 眼),随机分为术中应用丝裂霉素 C 的 36 例 (46 眼) 患者为 A 组,术中不用丝裂霉素 C 的 24 例 (32 眼) 为 B 组,分别观察术前、术后 1 个月和术后 3 个月两组眼压 (IOP)、角膜内皮细胞的密度 (CD)、平均细胞面积(AVG)及细胞面积变异系数(CV),分析其数量的改变及两组间的差异。**结果**:A 组术前眼压为 (35.4± 13.7) mmHg,B 组术前眼压为 (32.5± 13.5) mmHg 差异无统计学意义(P>0．05),A 组术后 1 个月及术后 3 个月眼压分别为 (15.7± 3.7) mmHg、(17.0 ± 3.2) mmHg,均低于 B 组的 (19.4± 3.7) mmHg、(20.2 ± 2.1) mmHg,差异有统计学意义 (P<0.05) 。A 组术前、术后 1 个月及术后 3 个月角膜内皮细胞密度分别为(2475± 484)个 / mm^2、(2199± 373)个 / mm^2、(2164± 332)个 / mm^2;平均细胞面积分别为(431.4± 67.6) μm^2、(480.6± 66.8) μm^2 、(463.8± 46.2) μm^2 细胞面积变异系数分别为(31.1± 7.4)%、(34.4± 6.3)%、(31.2± 7.5)% ;术后 1 个月及术后 3 个月各参数与术前比较,差异均有统计学意义(P< 0．05)。B 组术前、术后 1 个月及术后 3 个月角膜内皮细胞密度分别为(2342± 94)个 / mm^2、(2185± 215)个 / mm^2、(2074± 218)个 / mm^2 ;平均细胞面积分别为(453.9± 94.8)μm^2、(516.3± 100.8) μm^2、(499.81± 106.4) μm^2 细胞面积变异系数分别为(30.2± 3.0)%、(32.7± 2.9)%、(31.4± 4.3)% ;除术后 3 个月角膜内皮细胞与术前比较有意义 (P < 0.05)外,余参数术后 1 个月及术后 3 个月与术前比较差异均无统计学意义(P>0.05)。术后 1 个月 A 组的角膜内皮细胞丢失率为 10.4%高于 B 组的 6.1%，差异有统计学意义 (P<0.05)；术后 3 个月 A 组的角膜内皮细胞丢失率为 11.1%高于 B 组的 10.0%,差异无统计学意义(P>0.05)。**结论**:小梁切除术中用丝裂霉素 C 的降压效果比不用丝裂霉素 C 的效果好,但短期内前者角膜内皮细胞的丢失率高于后者。

关键词:角膜内皮细胞;丝裂霉素 C(MMC);小梁切除术

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