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·基础研究·

# 2种大鼠青光眼滤过手术模型在结膜抗瘢痕研究中的应用比较

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[摘 要] **目的** 比较前房穿刺法和植管法建立的2种大鼠青光眼滤过手术模型,并观察术后眼压和结膜滤过泡情况及术后结膜瘢痕形成过程。**方法** 将30只SD大鼠分为穿刺组和植管组,其中穿刺组15只15眼采用穿刺法,植管组15只15眼采用植管法。分别于术前及术后3 d、1周、2周、4周检测大鼠眼压,观察手术区结膜及角膜组织的充血水肿情况、前房情况及滤过泡形态变化,并记录滤过泡维持时间及生存率;术后4周分别摘取2组大鼠眼球制作石蜡切片,行HE染色、苦味酸狼红染色观察结膜切口的愈合情况及瘢痕形成。**结果** 2组术后3 d眼压均明显低于术前;2组平均眼压比相比,仅术后1周差异具有统计学意义($P<0.05$),术后3 d、2周、4周差异均无统计学意义($P>0.05$)。穿刺组术后1周滤过泡形态扁平,术后2周几乎观察不到;植管组术后1周滤过泡隆起较穿刺组明显,术后2周滤过泡逐渐局限,结膜组织包裹PE管末端,术后4周仅观察到滤过区结膜组织包裹PE管末端。穿刺组滤过泡维持时间为3~5 d,平均(3.5±0.64)d;植管组为10~14 d,平均(12.5±1.85)d。滤过术后1周植管组滤过泡的生存率(26.25±3.25)%高于穿刺组的(10.50±1.86)%,差异具有统计学意义($P<0.05$)。术后4周组织切片染色显示,2组手术区结膜组织均可见大量胶原组织沉积,而植管组术区结膜组织明显较厚。**结论** 大鼠前房穿刺法与植管法均可成功建立青光眼滤过手术动物模型,且植管法更有利于观察滤过泡形态及瘢痕形成,可用于青光眼术后抗瘢痕相关研究。

[关键词] 青光眼;滤过性手术;瘢痕;动物模型;大鼠

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## Application and comparison of two rat glaucoma filtering models in the conjunctival anti-scarring study

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**Abstract: Objective** To compare the establishments of two different models of glaucoma filtering surgery in rats by the anterior chamber puncturation and catheterization, and observe the postoperative intraocular pressure, conjunctival filtering blebs and postoperative conjunctival scar formation process. **Methods** Totally 30 SD rats were divided into the puncturation group and catheterization group, 15 rats with 15 eyes were included in the puncturation group and 15 rats with 15 eyes were included in the catheterization group. Intraocular pressure

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were measured before operation and 3 days,1 week,2 weeks and 4 weeks after operation. The hyperaemia and oedema of the conjunctiva and corneal tissue in the operation area, anterior chamber conditions, and morphological changes of the conjunctival filtration bleb were observed and the holding time and survival rate of filtration blebs were recorded. The eyeballs of rats in the two groups were taken and made into paraffin sections 4 weeks after surgery and the conjunctival incision healing and scar formation were observed by hematoxylin and erosin (HE) staining and picrosirius red staining. **Results** The intraocular pressures in both two groups 3 days after operation were significantly lower than those before operation. There was statistically significant difference in the mean intraocular pressure ratio 1 week after operation between the two groups ($P<0.05$), but no significant difference was found 3 days,2 weeks and 4 weeks after operation between the two groups ($P>0.05$). In the puncturation group, the shape of the filtration blebs was flat after 1 week, but almost no filtration bleb was observed after 2 weeks. In the catheterization group, the bulging filtration blebs were more obvious than those in the puncturation group but gradually limited 2 weeks after operation with the end of PE tube wrapped by conjunctival tissues, and only the conjunctival tissues in the filtering area were observed to wrap the end of the PE tube 4 weeks after operation. The holding time of the filtration blebs in the puncturation group was 3 to 5 days, with an average of (3.5 ± 0.64) days, and that in the catheterization group was 10 to 14 days, with an average of (12.5 ± 1.85) days. The survival rate of filtration blebs in the catheterization group 1 week after operation was (26.25 ± 3.25)%, which was higher than that in the puncturation group (10.50 ± 1.86)%, the difference was statistically significant ($P<0.05$). The tissue slices staining showed that a large amount of collagen tissues deposition was observed in the conjunctival tissue of the operation area in both two groups, while the conjunctival tissues in the catheterization group was significantly thicker. **Conclusion** Both anterior chamber puncturation and catheterization method can successfully establish the glaucoma filtering surgery animal model, and the catheterization method can be more conducive to observe the shapes and scar formation of filtration blebs and can be used for the anti-scarring study after glaucoma surgery.

**Keywords**: glaucoma; filtering surgery; scar; animal model; rat

青光眼滤过手术是药物控制不良的青光眼患者选择的主要治疗方式之一,术后滤过通道瘢痕形成是导致手术失败的主要原因[1-2]。如何预防及减少瘢痕的形成是研究热点之一,以往研究采用不同种属的动物和方法建立青光眼滤过手术模型,如兔、猴等,但兔眼的解剖和基因与人眼差异较大,猴虽与人的种属相近,但成本高、来源困难,限制了其应用[3-6]。鼠眼基因序列与人眼同源性高,以其建立青光眼滤过手术模型更利于研究应用。已有研究报道多种青光眼动物模型的制备方式,鼠逐渐成为青光眼滤过手术的主要动物模型之一[7-9]。不同于兔、猴等动物,鼠的眼球很小,无法完全模拟人青光眼滤过手术,常通过前房穿刺、结膜下注射、引流管植入等方法模拟青光眼滤过手术,但目前尚缺乏统一的滤过术模型建立方法。本研究拟通过前房穿刺法和前房植管法建立 2 种不同的大鼠青光眼滤过手术模型,比较其在青光眼术后抗瘢痕研究中的应用。

## 1 材料与方法

### 1.1 主要试剂及仪器

显微镜(Leica,德国),石蜡组织切片机(Leica,德国),0.4% 盐酸奥布卡因(参天制药有限公司,日本),10% 水合氯醛(生工,中国),静脉穿刺微管系统(Fine Science Tools,美国),粘弹剂(博士伦,美国),Direct red 80(Sigma,美国),Fast green FCF(Sigma,美国),Picric acid(Sigma,美国),HE 试剂盒(北京中杉,中国),倒置荧光显微镜(Leica,德国),数码照相系统(SPOT,美国),超低温冰箱(SANYO,日本),台式低温高速离心机(Heraus,德国),扫描分析软件系统(LabworksTM Analysis Software,美国),手术显微镜(苏州六六,中国),Tonolab 眼压计(Icare,美国),眼科手术显微器械(苏州医疗器械有限公司,中国)。

### 1.2 方法

雄性 SD 大鼠 30 只(由第三军医大学实验动物中心提供),体质量 200 ~ 250 g,SPF Ⅱ级。实验遵循《实验动物管理条例》,遵从《善待实验动物指导性意见》的相关伦理学规定。

1.2.1 大鼠滤过手术模型的建立[7,9] 以大鼠右眼为手术眼,术前使用 0.4% 盐酸奥布卡因滴眼液滴术眼 3 次,10% 水合氯醛腹腔注射麻醉,0.5% 碘伏溶液常规消毒术眼周围,0.25% 氯霉素滴眼液冲洗结膜囊 3 次。将大鼠随机分为穿刺组和植管组,其中穿刺组 15 只 15 眼采用穿刺法,以角膜缘为基底,制作结膜瓣约 2 mm × 3 mm,将 25G 针头于角膜缘平行虹膜面穿刺入前房,10-0 缝线缝合结膜瓣;植管组 15 只 15 眼采用植管法,同样制作结膜瓣,25G 针头穿刺入前房,植入静脉穿刺微管(PE 管)2 ~ 3 mm,一端植入前房,另一端埋于结膜下,于角膜缘固定缝合 PE 管 1 针,10-0缝线缝合结膜瓣。术后氯霉素滴眼液点术眼。

1.2.2 术后眼压及滤过泡形态观察 分别于术前及术后3 d、1周、2周、4周使用iCARE眼压计测量并记录眼压,平均眼压比=术后眼压/术前眼压×100%。观察手术区结膜及角膜组织的充血水肿情况、滤过泡形态、前房情况等,若大鼠结膜感染或前房出血、感染等,则认为模型建立失败,每组建模成功10只以上。通过前节拍照观察结膜滤过泡形态变化,记录滤过泡维持时间及生存率。采用Image J 1.8.0软件分析滤过泡面积,分别计算术后3 d及术后1周滤过泡的面积。滤过泡生存率=术后1周滤过泡面积/术后3 d滤过泡面积×100%,每组至少8只大鼠,计算后取平均值。

1.2.3 组织学观察 于术后4周分别处死2组大鼠,摘取大鼠眼球置于眼球固定溶液(4%多聚甲醛、乙酸、冰醋酸)中固定24 h。平行视轴切去眼球的1/3,注意保护手术区域,继续固定12 h,55%乙醇放置过夜,梯度乙醇(60%、70%、80%、90%、100%)脱水,二甲苯透明5 min,浸蜡1 h,进行石蜡包埋,连续石蜡切片(5 μm),37 ℃烤片过夜。石蜡切片分别行苏木精-伊红(HE)染色、苦味酸狼红染色以观察结膜切口的愈合情况及瘢痕形成。

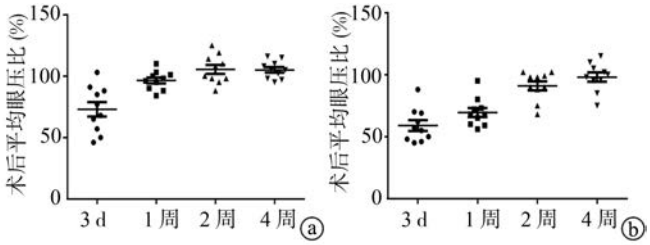
1.3 统计学方法

采用SPSS 16.0软件进行统计分析,数据经*Shapiro-Wilk*检验呈正态分布,经*Levene*检验证实方差齐。计量资料用均数±标准差($\bar{x} \pm s$)表示,2组间比较采用$t$检验,多组数据间比较采用重复测量的单因素方差分析one-way ANOVA,以$P<0.05$为差异具有统计学意义。

## 2 结果

2.1 大鼠滤过手术模型眼压变化情况

因实验观察中发生死亡或后期发生感染等导致部分模型失败,完整模型每组10只。每个时间点测量10只大鼠,2组术后3 d眼压均明显低于术前;穿刺组术后3 d至1周眼压逐渐升高至术前水平,植管组术后2周眼压逐渐升高至术前水平。穿刺组术后3 d、1周、2周、4周平均眼压比分别为(73.01±18.89)%、(96.50±7.69)%、(105.51±11.65)%、(105.34±7.28)%,见图1a;植管组术后3 d、1周、2周、4周平均眼压比分别为(59.00±13.57)%、(69.61±11.48)%、(88.03±11.55)%、(98.10±11.61)%,见图1b。仅术后1周2组平均眼压比差异具有统计学意义($P<0.05$),术后3 d、2周、4周差异均无统计学意义($P>0.05$)。




a:穿刺组;b:植管组 平均眼压比=术后眼压/术前眼压×100%

**图1 大鼠滤过手术后各时间点平均眼压比**

2.2 大鼠滤过手术后滤过泡形态变化

穿刺组大鼠术后3 d手术区结膜充血水肿,穿刺口角膜稍水肿,但滤过泡较扁平;术后1周形态扁平;术后2周、4周手术区结膜组织无明显充血,几乎观察不到滤过泡,前房同术前比较无明显变化,晶状体无明显浑浊(图2)。植管组大鼠术后3 d角膜穿刺口稍水肿,引流管在位,未接触晶状体,滤过泡隆起弥散,结膜组织充血水肿,前房可见少许渗出,部分少许积血,晶状体无明显浑浊;术后1周角膜透明,引流管在位,滤过泡隆起较穿刺组明显;术后2周滤过泡逐渐局限,结膜组织包裹PE管末端,结膜表面血管化;术后4周仅观察到滤过区结膜组织包裹PE管末端(图2)。穿刺组滤过泡维持时间为3~5 d,平均(3.5±0.64)d;植管组为10~14 d,平均(12.5±1.85)d。滤过术后1周植管组滤过泡的生存率(26.25±3.25)%高于穿刺组的(10.50±1.86)%,差异具有统计学意义($P<0.05$,$t=4.205$),见图3。

2.3 大鼠滤过泡组织HE染色及苦味酸狼红染色结果

术后4周,观察2组手术区结膜组织切片HE染色。穿刺组可见球结膜组织增厚,中性粒细胞、单核巨噬细胞浸润,成纤维细胞增殖。植管组手术区放置PE管,切片可见管囊腔样结构,结膜组织仍有明显的炎症反应,大量中性粒细胞、单核巨噬细胞浸润,成纤维细胞增殖较穿刺组明显。苦味酸狼红染色显示手术区的胶原组织沉积情况,2组滤过手术区球结膜下均可见红色胶原纤维沉积,PE管被结膜下致密的胶原组织包裹,未见明显的滤过泡结构(图4)。

## 3 讨论

滤过手术是治疗青光眼的主要方式之一,功能性滤过泡的瘢痕形成是手术失败的主要原因,如何维持滤过功能、减少或者延缓瘢痕的形成是研究的重点及难点。本研究通过观察和对比前房穿刺法和植管法建立的2种大鼠青光眼滤过手术模型,观察眼压、滤过泡的形态与组织学改变,探讨不同手术模型在青光眼滤

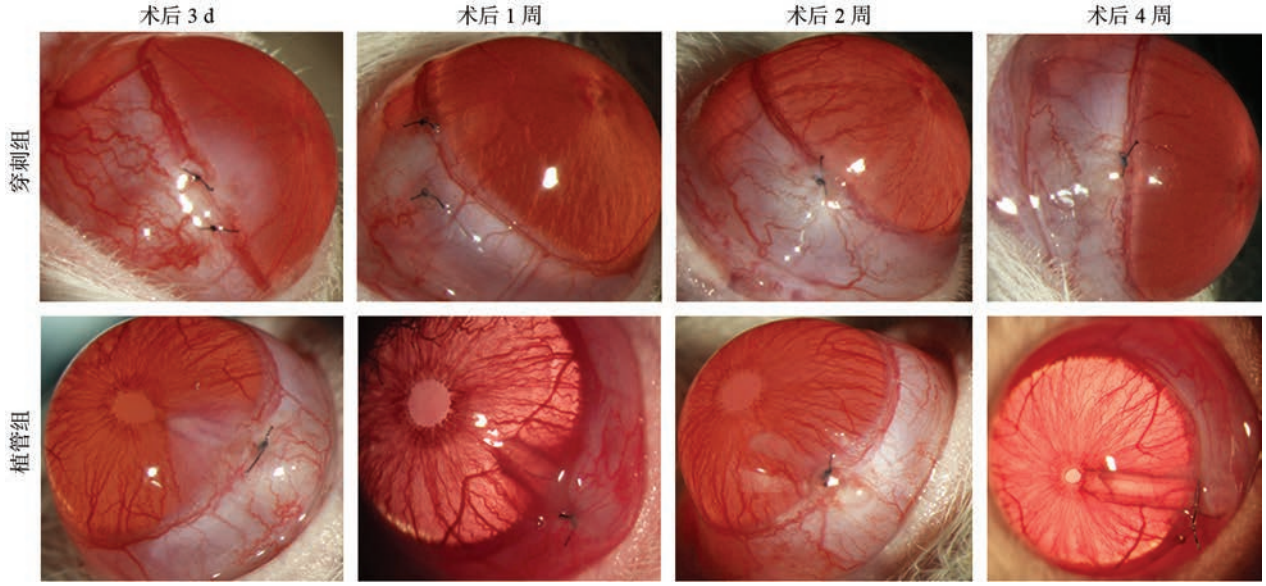



**图 2 大鼠滤过手术后滤过泡形态变化**

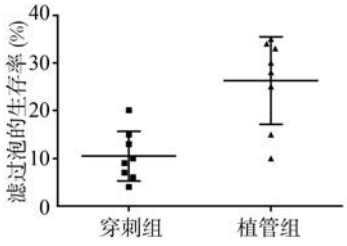



**图 3 大鼠滤过手术后 1 周滤过泡的生存率**

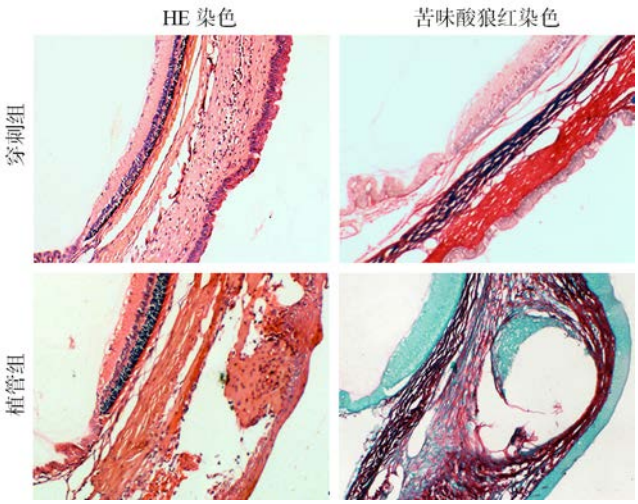



**图 4 术后 4 周大鼠手术区组织 HE 染色及苦味酸狼红染色(×100)**

过手术后结膜瘢痕形成研究中的应用。

模拟抗青光眼手术的模型可采用不同种属的动物和方法建立,其中最常用的是兔子和鼠。兔子眼球较大,比较适合建立动物研究的手术模型,易于手术操作和术后观察,但兔眼的解剖结构和基因与人眼相比有很多不同[5,10]。在非灵长动物中,鼠的眼球前房结构与人最相似,具有小梁网和 Schlemm 管结构,且鼠基因序列与人类相似度高、成本较低,是模拟抗青光眼手术的理想模型[11-13]。Reichel 等[14]首次使用小鼠模拟青光眼滤过手术模型,使用 27G 针头结膜下注射 25 μL PBS,术后 1 ~ 3 d 小鼠出现炎症反应和成纤维细胞增生,7 ~ 14 d 可观察到结膜瘢痕的形成。Seet 等[8]采用穿刺法构建小鼠青光眼滤过手术模型,结果显示术后 14 d 形成典型的瘢痕,丝裂霉素 C 处理后可延长滤过泡生存时间至 28 d。Sherwood 等[11]利用 30G 导管制作大鼠青光眼滤过手术模型,认为穿刺法滤过通道维持时间过短,不利于观察瘢痕形成过程。本研究将 2 种手术方式进行对比,发现穿刺法操作简单,但穿刺通道太小,容易闭合,术后滤过泡不明显且容易消失,不利于观察滤过泡形态;而植管法通过微管将房水引流至结膜下,滤过泡维持相对稳定,但手术操作较为困难,容易损伤晶状体及虹膜,引起前房出血、引流管移位甚至脱落,因此植管法需要一定的操作经验。术后测量大鼠眼压观察滤过泡形态变化,结果显示植管法大鼠眼压普遍较穿刺法低,且滤过泡形态维持时间更长。

明佩佩等[15]采用前房引流管(硅胶管:内径 0.3 mm、外径 0.6 mm)植入术制作大鼠眼球结膜滤过泡模型,发现术后 1 周左右是结膜切口的愈合期,滤过泡生存时间为 7 ~ 17 d,平均(11.93 ± 2.23)d,术后2 周内结膜瘢痕形成最为关键,滤过泡生存率下降至 20%,手术区结膜下成纤维细胞增生活跃,胶原纤维沉积,导致滤过泡瘢痕化。本研究中术后 1 周植管组滤过泡生存率为(26.25 ± 3.25)%,分析原因可能与植入材料、手术操作、动物本身等相关;植管组组织石蜡切片苦味酸狼红染色结果显示,手术区结膜下可见致密的胶原纤维沉积,提示手术后

1~2 周是瘢痕形成的关键期。

目前引入一种新的青光眼引流管微创植入装置(XEN 凝胶引流管:长 6 mm,管内径约 45 μm),这种新型的引流管手术更微创,术后反应更轻,生物相容性好,但术后仍面临结膜瘢痕形成导致手术失败等问题,第一代产品在不使用抗代谢药物的情况下术后失败率为50%~80%[16-17]。Vera 等[18]认为加强青光眼患者手术前后抗炎治疗,减少结膜组织炎症反应,使用抗代谢药物有助于减少结膜瘢痕形成。如何预防和减少术后结膜瘢痕形成仍然是目前青光眼手术治疗的一大难题,本研究中在前房植入 PE 管以引流房水、降低眼压,与 XEN 凝胶引流管手术原理类似,可为相关药物研究提供实验基础。

本研究选择大鼠作为青光眼滤过手术后滤过泡瘢痕化研究的动物模型,采用前房穿刺法和植管法模拟青光眼滤过手术。2 种方法均可形成结膜滤过通道,与穿刺组相比,植管组术后眼压更低,滤过泡形态明显,术后 1 周滤过泡的生存率更高,利于术后观察瘢痕形成的过程,可为青光眼术后抗瘢痕干预提供较理想的动物模型。

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