

不同缝合方式对大鼠角膜穿透伤后内皮细胞影响的研究

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摘要 目的 通过手术缝合治疗大鼠角膜穿透伤,探索全层缝合、深板层缝合、及不缝合对角膜内皮细胞的影响。方法 建立大鼠角膜穿透伤模型,同一手术者对角膜切口进行全层、深板层、及不缝合操作。在裂隙灯下动态观察角膜创伤愈合情况,对不同时间点愈合角膜行内皮细胞台盼蓝-茜素红联合活细胞染色及HE染色,观察内皮细胞损伤、修复及白细胞浸润情况。结果 无论是全层缝合还是板层缝合以及不缝合组角膜内皮细胞均损伤明显。但从第1天观察至1月,三组损伤区面积大小无明显差别。结论 全层和深板层缝合及未缝合组可直接造成角膜内皮细胞受损,继发性炎症反应损伤后角膜内皮细胞的损害,伤口周围1.5 mm处角膜内皮几乎损失殆尽,内皮细胞受损可使角膜损伤区水肿迁延不愈,最终形成瘢痕愈合,所以角膜内皮损伤及最终愈合程度三组间无明显差异。

关键词 台盼蓝 茜素红 角膜 内皮细胞

中图分类号 R779.12 R779.62 文献标识码 A 文章编号:1673-6273(2012)21-4027-04

The Rat Cornea after Penetrating Injury of Different Suture on Corneal Endothelium Injury Research

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ABSTRACT Objective: To investigate the effect of the different suture patterns after rat corneal endothelium injury by the operation of suture in the treatment of rat corneal penetrating injury. **Methods:** The rat corneal penetrating injury model was built by the same surgical, and suture was divided into: full-thickness, 90% thickness and non-suture, and the model was observed the corneal wound healing in the slit lamp. Corneal endothelium cells were stained at different time points by trypan blue and alizarin red and HE. And then we observed the endothelial cell damage, repair, and leukocyte infiltration. **Results:** Corneal endothelium injury was obvious in the different suture patterns. However, the size of the damage area was no significant difference in three groups from one day to 30 days. **Conclusions:** The three groups make the corneal endothelial cell damage, and the corneal endothelium is almost wiped out around the wound 1.5mm. Endothelial cell injury makes corneal injury delayed healing. Finally, the scar was formed. Corneal endothelial damage and healing between the three groups had no significant difference.

Key words: Trypan blue; Alizarin red; Cornea; Endothelial cells

Chinese Library Classification (CLC): R779.12 R779.62 **Document code:** A

Article ID:1673-6273(2012)21-4027-04

前言

尽管眼睛只占全身表面积的0.1%,只占人身前表面的0.27%,但是它对于个体以及社会是非常重要的,人类绝大多数信息通过视觉得到。而角膜是组成屈光介质的重要结构,位于眼球的最前部,在受到外力作用后,更容易遭受损害。故角膜穿透伤是眼外伤中最常见的,因为眼的屈光力大多由角膜提供,所以识别和治疗角膜损伤是恢复视力的关键。手术缝合角膜裂伤后的瘢痕直接影响术后视力,目前利用显微缝合技术,对角膜组织的损伤小,瘢痕小,可使伤口达到屈光度良好的对合,已得到大家共识。但角膜缝合时深度的掌握,近年来却有分歧,传统观点认为全层缝合会损伤角膜内皮,而深板层缝合则不会,

但缝合全层角膜后会对角膜内皮带来更大的损伤,一直以来,未见相关实验验证。本研究着重探讨全层缝合和深板层缝合方式到底对角膜内皮损伤有无明显区别,为临床应用提供依据。

1 材料与方法

1.1 动物模型与制作

选健康SD大鼠48只(购自西安交通大学实验动物中心),大鼠均为10周大小,雌鼠和雄鼠各一半,体重大约为250-280 g。将所有大鼠分为4组,1组(全层缝合角膜伤口, n=15), 2组(板层缝合角膜伤口, n=15), 3组(不缝合角膜伤口, n=15), 4组(正常对照组 n=3)模型制作方法及分组同前期研究。

动物模型的制备 每只大鼠用10%的水合氯醛腹(扬州市奥鑫助剂厂 批号 20020314)腔注射麻醉,而且左眼作实验组,右眼作对照组,在腹腔麻醉后,再用0.4%盐酸奥布卡因(日本参天制药株式会社 批准文号 H20020318)行表面麻醉。将大鼠放置于

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(收稿日期 2012-03-18 接受日期 2012-04-14)

试验台显微镜下,用小型开睑器分开大鼠眼睑,12点处行上直肌牵引。从角膜缘3点处起至9点处行长约4-5mm角膜横行穿通裂伤。手术过程:将组组造模后的大鼠用托百士眼液(爱尔康中国眼科产品有限公司批准文号:H20091082)冲洗后,用ALCON10-0尼龙缝线分别缝合3~4针,组为全层深度缝合角膜,组为深度为90%缝合角膜,组不缝合角膜。术后均给予局部托百士眼液抗感染治疗。分别取1天、1周、1月大鼠角膜进行裂隙灯及行台盼蓝(上海雅吉生物科技有限公司批号20021031)-茜素红(上海雅吉生物科技有限公司批号20021031)联合活细胞染色检测角膜内皮细胞观察并行角膜HE染色的病理切片进行对比,还可应用前节OCT进行分析。

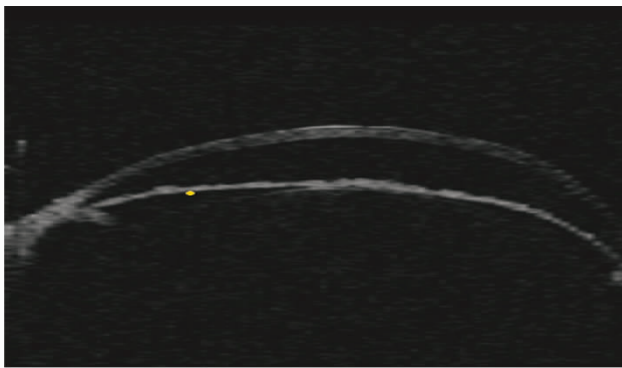


图1 未缝和7天时 OCT 检查

Fig.1 The group of no suture in OCT examination for the 7th

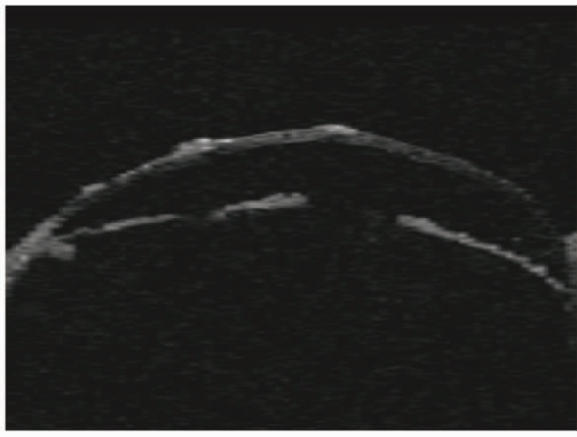


图2 板层缝合7天时 OCT 检查

Fig.2 The group of 90%-thickness suture in OCT examination for the 7th

1.2 前节 OCT 观察缝线位置及前房情况

于造模后1天、3天、7天、1月,在观察内皮前实验组行前节OCT(OCT光学相干断层成像 Heidelberg 德国)检查缝线位置及前方深度及有无感染。

1.3 裂隙灯显微镜观察

于造模后1天、7天、1月,在进行内皮细胞染色前实验组以裂隙灯显微镜(苏州医疗仪器厂)下观察角膜伤口的愈合表现,确定前房深度正常,角膜伤口对合良好无感染迹象,角膜水肿程度。

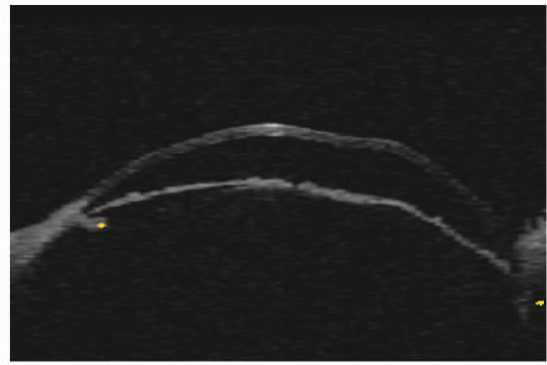


图3 全层缝合7天时 OCT 检查

Fig.3 The group of full-thickness suture in OCT examination for the 7th

1.4 角膜内皮细胞染色

在造模后各个时间点每个点抽取3只大鼠,以上述麻醉方法麻醉后裂隙灯检查角膜愈合情况及前房深度,确认无虹膜粘连后在显微镜下沿角膜缘环形剪下角膜,并注意不损伤角膜表面,涂粘弹剂(上海奇胜化学制剂厂)保护内皮,放置于玻璃皿中。将配置好的0.25%台盼蓝溶液滴于大鼠角膜片的内皮面上,保留2min,用眼用平衡盐(天津晶明新技术开发有限公司)冲洗,并用滤纸吸除多余的水分;然后用配置好的0.2%茜素红溶液滴于大鼠角膜片的内皮面上,保留3min,继续用眼用平衡盐冲后用2.5%中性戊二醛溶液滴于大鼠角膜的内皮面上,30分钟后将大鼠角膜片分别沿伤口区和垂直平分于伤口区均分为四份扇形,且未完全离断,然后在光镜下($\times 400$)观察伤口区及周边区域角膜内皮情况并拍照。

1.5 病理学观察

在造模后各个时间点进行完角膜内皮染色检查后将角膜用眼用平衡盐冲洗后用甲醛固定,角膜切片后行HE染色,在光镜下观察角膜穿通伤区及周边区域白细胞浸润程度及范围。

1.6 内皮细胞损伤程度

应用Image2Pro Plus 4.5图像处理软件将在光镜下($\times 400$)拍到的伤口区及周边区域的角膜内皮照片输入计算机中,图中如果角膜内皮细胞损伤,则角膜内皮细胞核被台盼蓝染为蓝色,而未损伤的角膜内皮细胞仅细胞壁染为淡红色,此软件可标出角膜损伤内皮细胞蓝染区域,并可测量出该区域的面积,然后计算角膜内皮损伤面积与角膜面积的比例,每组每观测点取6只眼球的平均值,然后用来比较3组间角膜内皮缺损面积,以判断有无差异。

1.7 统计学分析

各组数据以 $\bar{X} \pm S$ 表示,采用SPSS13.0统计软件进行单变量方差分析和均数间的多重比较。

2 结果

2.1 角膜内皮的光镜观察结果

正常角膜内皮为单层六角形内皮细胞构成,细胞为边界清晰,大小基本均匀一致,核位于中心。角膜穿通伤后一天观察,不同缝合组沿伤口周围2mm处均未见正常内皮细胞且损伤区可见大量蓝染的细胞核,2mm以外均可见正常染色的六边形细胞,交界处细胞变形不规则,但未见细胞核染色,7天观察,不

同缝合组损伤区内仍未见正常角膜内皮细胞,伤口 2 mm 外细胞形态增大,交界处皮细胞变形,变长、增大,向损伤区内移行;1 月观察,不同缝合组角膜损伤区瘢痕形成,瘢痕周边角膜内皮细胞较正常细胞大,角膜周边内皮细胞仍呈交错排列,排列紧密整齐。

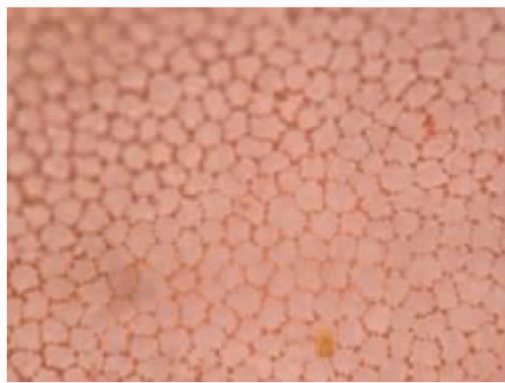


图 4 未损伤区角膜内皮(台盼蓝-茜素红联合染色×400)

Fig.4 The zone of corneal endothelium were not damaged



图 5 未缝合后 1 天伤口区角膜内皮细胞全部死亡(台盼蓝-茜素红联合染色×400)

Fig.5 No suture wound area of corneal endothelial cells of all death



图 6 深板层缝合后 1 天伤口区角膜内皮细胞全部死亡(台盼蓝-茜素红联合染色×400)

Fig.6 Days after 90%-thickness suture wound area of corneal endothelial cells of all death



图 7 全层缝合后 1 天伤口区角膜内皮细胞全部死亡(台盼蓝-茜素红联合染色×400)

Fig.7 The 90%-thickness suture after 1-day of wound area of corneal endothelial cells all died

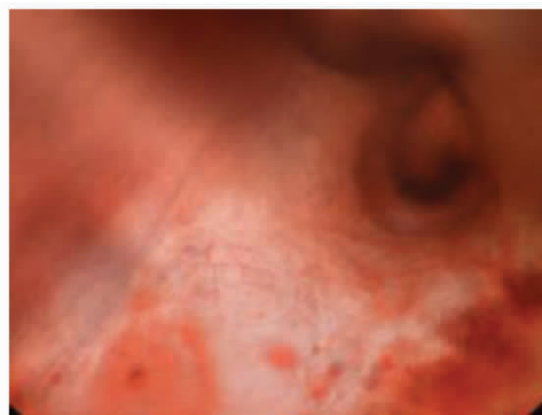


图 8 缝合后 1 月伤口区形成瘢痕无正常内皮细胞(台盼蓝-茜素红联合染色×400)

Fig.8 One month after suture the wound area forming scar without normal endothelial cells

2.2 HE 染色

HE 染色显示、组损伤后各时相点角膜白细胞数比较,全层缝合的角膜组织炎症反应时间较长,白细胞数较其他两组多。

2.3 角膜内皮台盼蓝-茜素红活细胞联合染色观察

在完成模型制造后伤口缝合后即刻各组均行角膜内皮染色三组均可见损伤区内皮面均为蓝染的细胞核,且核肿胀、模糊,茜素红不着色,周边 2mm 外可见正常的六边形细胞。角膜穿透伤后一天观察,不同缝合组沿伤口处均未见正常内皮细胞且损伤区可见大量蓝染的细胞核,2mm 以外均可见正常染色的六边形细胞,交界处细胞变形不规则,但未见细胞核染色,7 天观察,不同缝合组损伤区内仍未见正常角膜内皮细胞,伤口 2mm 外细胞形态增大,交界处皮细胞变形,变长、增大,向损伤区内移行;1 月观察,不同缝合组角膜损伤区形成纤维条索,纤维条索周边角膜内皮细胞较正常细胞大,角膜伤口周边内皮细胞体积均增大但仍呈交错排列,排列紧密整齐。通过观察,三组间在不同时相内皮损伤区面积大小无没有统计学意义 $P>0.05$ (见表 1),故没有显著差异。

表 1 角膜内皮损伤区不同时间占角膜面积的比例(S)%

Table 1 The corneal endothelial damage zone at the all times to account for corneal area ratio(S)

Group	n	Time(days)			
		1	3	7	30
	6	18.47± 0.43	14.75± 0.32	10.57± 0.52	6.10± 0.39
	6	18.53± 0.41	15.11± 0.36	10.54± 0.56	6.13± 0.36
	6	18.37± 0.29	14.35± 0.38	10.58± 0.53	6.17± 0.40

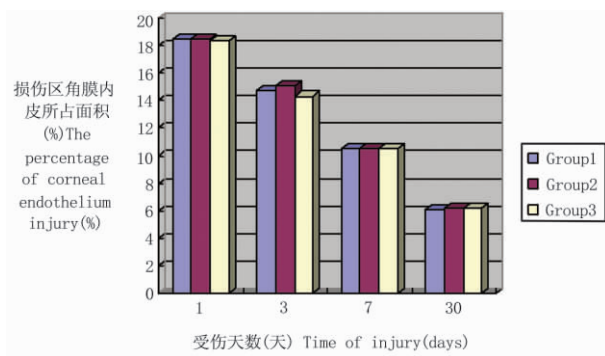


图 8 角膜内皮损伤区不同时间占角膜面积的比例

Fig.9 Corneal endothelial damage zone at the all times to account for corneal area ratio

3 讨论

眼外伤是常见的多发性眼病,是视力损害的主要原因,在单眼盲中居首位。角膜位于体表,是眼球的最前部,因此极易遭受外伤损害。故角膜穿通伤是眼外伤中最常见的。由于角膜中央没有血管,角膜伤口愈合缓慢,且不会形成肉芽组织,外伤后如果要恢复角膜的正常功能,需具备:无血管、角膜基质层精确排列、角膜前后表面重建、基质层正常张力恢复。而其中角膜前后表面重建尤为重要,早期快速而准确的治疗方法是可以使损伤及时得到控制并向好的方向转化,同时减少并发症。角膜穿通伤的治疗主要是手术修复,手术的关键步骤就是缝合伤口,手术缝合角膜裂伤后的瘢痕直接影响术后视力,目前利用显微缝合技术,对角膜组织的损伤小,瘢痕小,可使伤口达到屈光度良好的对合,已得到大家共识。但角膜缝合时缝线的深度,及缝合时深度的掌握,缝合全层角膜后会对角膜内皮带来更大的损伤吗,一直是难点所在。

角膜内皮是角膜基质和房水之间的通透屏障,角膜内皮是主要负责将角膜基质中的水分泵出的六角形细胞组成,因此保证了角膜的脱水状态从而维持角膜的透明性。角膜内皮来源于神经嵴,只可以有限的再生,所以它们极其脆弱,任何刺激比如:创伤、炎症时角膜通透性将增加,严重时角膜内皮大量破坏,当有足够数目的细胞失功和内皮细胞密度下降到临界水平以下时,即可发生角膜水肿改变。使角膜混浊。谢立信等^[6]发现,内皮细胞的损伤可导致角膜水肿,内皮细胞丢失越多,角膜水肿越重,二者具有对应关系,故更应尽量减少对内皮细胞的损伤,以维持角膜的透明性。

本实验中,造模后角膜穿通伤后一天观察,不同缝合组沿伤

口周围 2 mm 均未见正常内皮细胞且损伤区可见大量蓝染的细胞核,2 mm 以外均可见正常染色的六边形细胞,交界处细胞变形不规则,但未见细胞核染色,故可用角膜损伤内皮细胞蓝染区域的面积,来比较 3 组间角膜内皮缺损面积,以判断有无差异。本研究提示:不管是全层缝合还是深板层缝合或者是不缝合,损伤区角膜内皮都消失殆尽,在治疗时一定要采取一些积极有效的措施,一方面保护内皮细胞免受继发性损害,另一方面使伤口对合良好时期瘢痕尽量最小化。最终为最大保留视力创造有利条件^[5]。所以在处理角膜穿通伤时应根据情况,尽量使角膜愈合瘢痕小,保留相对多的视力,而且要使炎症反应轻,最终还要尽可能保护内皮,以维持正常的角膜透明性,保留最好的视力。

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