·工作综述·

血管化自体下颌下腺移植治疗重症干眼20年研究

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[关键词] 下颌下腺;干眼;干燥性角结膜炎;移植,自体;泪器疾病
[中图分类号] R781.7 [文献标志码] A [文章编号] 1671-167X(2018)01-0001-04
doi:10.3969/j.issn.1671-167X.2018.01.001

干眼是泪膜异常导致的以角结膜干燥为主的眼表疾病,重症患者因眼干、视力减退甚至失明而严重影响生活质量。1998年以来,北京大学口腔医学院、基础医学院和首都医科大学北京同仁医院组成联合项目组,开展血管化自体下颌下腺移植治疗重症干眼的基础与临床紧密结合的系列研究,将患者自体下颌下腺游离后移植到颞部,相应血管进行吻合,分泌导管转移到眼眶,用下颌下腺分泌的唾液替代泪液,术后采用多种措施,人工调控移植腺体的分泌,取得了良好的治疗效果。

1 系统改进血管处理技术,提高移植下颌下腺的成活率

下颌下腺移植属于头面部的小器官移植,手术成功的基本要素是腺体成活和导管通畅,吻合血管畅通常是保证腺体成活的关键。下颌下腺的主要回流静脉变异性大,部分患者颞部受区静脉管径纤细、管壁菲薄,常与下颌下腺静脉管径不匹配,吻合后易出现静脉回流障碍,形成血栓,是影响手术成功的难点。我们应用增强CT静脉成像技术,术前预测下颌下腺静脉的变异以及面前静脉与颞浅静脉管径是否匹配[1]。术中采用"3步法"判断下颌下腺的主要回流静脉,即:钳夹静脉,观察其充盈状态;切断静脉,观察渗血状态;腺体游离后,经面动脉灌注肝素盐水,观察静脉渗出液,提高了判断主要回流静脉的准确率[2]。对颞部静脉过细的患者采用血管端侧吻合技术、腺体静脉缩窄技术以及前臂头静脉搭桥,桥接下颌下腺静脉及颈外静脉,明显降低了术后血管危象的发生率,使移植腺体的成活率达到92.5%[1-2]。

2 采用部分下颌下腺移植新术式,有效预防或减轻术后泪溢

传统下颌下腺移植术为整体腺体移植,术后60%患者出现移植腺体过度分泌而导致泪溢,明显影响患者的生活质量,需进行二次或多次减量手术治疗[3]。我们通过制作下颌下腺血管和导管铸型模型,明确下颌下腺的动静脉和导管系统以腺叶为单位,互相并行,呈树枝状分布,为以腺叶为单位削减腺体的部分下颌下腺移植提供了解剖学基础[4]。在家兔实验性部分下颌下腺移植腺体全部成活的基础上,对术前下颌下腺体积大、功能好、预期术后出现严重泪溢的39例患者的42侧患眼实施了部分下颌下腺移植术,经与相同条件的整体下颌下腺移植术相比较,部分下颌下腺移植术可明显减轻泪溢程度,术后行二次减量切除手术的比例由81.8%下降为30%[5-6]。

3 完成世界上病例数最多的一组临床研究,充分证实下颌下腺移植治疗重症干眼的有效性

我们课题组自1999年以来,采用下颌下腺移植治疗193例患者的211侧重症干眼,是世界上病例数最多的一组临床研究。90%患者的腺体成活且导管保持通畅。在2014年以前治疗的患者中,163侧

基金项目:"十一五"国家科技支撑计划(2007BAI18B11)、国家自然科学基金(30730102、30171016、3034006、30371545、30640093、80170847、81470756、81271161、81170974、30700949、81300893)资助 Supported by the National Key Technology R&D Program for the 11th Five-Year Plan (2007BAI18B11) and the National Natural Science Foundation of China (30730102, 30171016, 3034006, 30371545, 30640093, 80170847, 81470756, 81271161, 81170974, 30700949, 81300893)
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网络出版时间:2017-12-19 16:03:28 网络出版地址:http://www.cnki.net/kcms/detail/11.4691.R.20171219.1603.022.html

患眼经1年以上随访,患者怕风、畏光的症状明显减轻,眼干有效缓解,可停用或很少使用人工泪液,眼表结构明显改善。56.3%的患者视力有不同程度提高,患者主观满意率达87.7%,实践证明下颌下腺移植是重症干眼的有效治疗手段。在积累丰富临床经验的基础上,由项目组牵头,制定了《血管化自体下颌下腺移植治疗重症角结膜干燥症指南》,发表于《中华口腔医学杂志》[7]。

4 揭示调控正常及移植下颌下腺唾液分泌的机制,提出人工调控移植腺体分泌的策略

正常下颌下腺的分泌受交感和副交感神经支配,移植术后由于腺体失神经支配,分泌机制改变,术后3个月内"休眠期"分泌过少易出现导管阻塞,术后6个月腺体分泌逐渐稳定,但部分患者因分泌过多出现泪溢[2-3]。针对这些临床问题,我们开展了调控正常及移植下颌下腺唾液分泌机制的系列基础研究,为人工调控移植腺体的分泌,全面提高下颌下腺移植治疗重症干眼的近、远期疗效奠定基础。

4.1 揭示调控下颌下腺分泌的新途径

4.1.1 首次提出激活辣椒素受体是调控下颌下腺分泌的新途径[8] 我们的研究在人、家兔和大鼠下颌下腺中发现了辣椒素受体的表达并明确了其分布特点[8-10]。采用离体动物下颌下腺灌流和健康志愿者局部涂抹辣椒素霜剂证实,激活辣椒素受体具有促进唾液分泌的功能[9-11]。机制研究揭示,激活辣椒素受体通过增加细胞内Ca^{2+}浓度调控水通道蛋白介导的跨细胞转运[12],而特异性敲低及过表达研究揭示occludin是构成下颌下腺紧密连接的重要功能蛋白和介导辣椒素经细胞外调节蛋白激酶(extracellular regulated protein kinase, ERK)1/2调控旁细胞途径通透性的关键分子[13-14]。

4.1.2 证实下颌下腺是脂联素的新来源和作用靶点 脂联素是调控能量代谢和免疫功能的脂肪因子。我们的研究结果证实,下颌下腺细胞可生成和分泌脂联素,并经激活腺苷酸活化蛋白激酶(AMP-activated protein kinase, AMPK)调控紧密连接蛋白claudin-4丝氨酸199位点的磷酸化及亚细胞分布,促进唾液分泌[15-16]。

4.1.3 揭示激活毒蕈碱乙酰胆碱受体(M受体)经调控紧密连接蛋白促进下颌下腺分泌的机制 既往对M受体调节紧密连接蛋白复合体的作用并不了解,我们的研究明确了激活M3受体可经ERK1/2/β-arrestin2/clathrin/ubiquitin途径下调紧密连接蛋白claudin-4的表达及分布,还可经MLC2/F-actin途径调控下颌下腺血管内皮紧密连接蛋白claudin-5的分布,进而增加血管的通透性,是促进唾液分泌的新机制[17-18]。这些研究从分子水平揭示了促下颌下腺分泌的网络调控机制,为治疗下颌下腺分泌功能低下,特别是帮助移植术后腺体顺利度过"休眠期"提供了新的思路。

4.2 明确促分泌神经介质和主要受体信号通路发生改变是导致下颌下腺分泌异常的重要机制

在下颌下腺移植的家兔模型中,"休眠期"腺体中M受体和辣椒素受体等的表达均明显降低,受体后信号通路功能抑制。分别给予卡巴胆碱和辣椒素等受体激动剂可上调相应受体的表达,减轻腺体萎缩,增加分泌量[12-13,19-21]。在下颌下腺移植术后出现泪溢的患者以及家兔模型中,M3受体含量增加,ERK磷酸化导致的F-actin重排参与了移植腺体分泌的增加[22-23]。对移植腺体分泌液进行蛋白质组学分析,明确了移植腺体分泌液蛋白成分的变化规律[24-25],并揭示A型肉毒毒素调控水通道蛋白转位的机制[26-27],这些研究揭示了促分泌的神经介质以及主要受体信号通路改变是导致移植腺体分泌异常的重要机制,为人工调控移植腺体的分泌奠定了实验基础,提出了以受体和紧密连接蛋白为靶点调控下颌下腺分泌的策略[8]。

5 创建术后调控移植下颌下腺分泌的体系

我们采用转化医学的模式,将基础研究结果应用于临床,对"休眠期"及出现泪溢的移植下颌下腺分泌进行人工调控,并制定了相应的临床规范。

5.1 提出"休眠期"辣椒素和卡巴胆碱联合用药的临床规范

通过研究,我们提出在"休眠期"给予辣椒素和卡巴胆碱的联合用药方案[28],其中,辣椒素霜剂涂抹在移植腺体皮肤表面,方法简便、安全,药效温和,患者自行在"休眠期"内用药,使腺体保持一定量的持续性分泌。卡巴胆碱作用强,由医生在"休眠期"间断注射,起到导管"内冲洗"的作用,两者作用互补。对下颌下腺移植术后115例患者的128侧患眼的随访结果显示,联合用药显著提高了移植腺体的分泌量,术后导管阻塞的发生率由18.18%降至6.25%[29-30]。

5.2 提出个性化泪溢防治的临床规范

我们对乙酰胆碱受体抑制剂阿托品凝胶进行了改良,增加其透皮性,并将其用于"泪溢"的控制。研究结果显示,移植下颌下腺皮肤表面涂药后,分泌量减少,泪溢症状减轻,患者主观舒适度提高,作用

时间持续约 3 ~ 5 h,适合于中度泪溢患者[31]。采用 A 型肉毒毒素在移植腺体局部注射,可有效减轻泪溢症状,作用持续 3 ~ 6 个月[27]。在此基础上,我们制订了下颌下腺移植术后"泪溢"防治的临床规范:(1)对于腺体体积大、功能好、术前判断可能出现严重泪溢的患者,采用部分下颌下腺移植术;(2)对于静止和刺激状态下均出现明显"泪溢"者,采用移植腺体部分削减术;(3)对于季节性、气温升高或运动刺激才引发"机会性泪溢"者,联合使用 A 型肉毒毒素和阿托品凝胶,其中 A 型肉毒毒素入夏时使用一次,控制季节性"泪溢",阿托品凝胶日常备用,控制运动刺激性"泪溢";(4)对于明显运动刺激才引发的"机会性泪溢"者,单独使用阿托品凝胶[29]。

6　建立诊治慢性阻塞性移植下颌下腺炎的新技术

6.1　提出慢性阻塞性移植下颌下腺炎的疾病概念和诊断标准

下颌下腺移植术后,少数患者分泌液持续量少而黏稠,移植腺体反复肿胀,有的甚至发生导管完全阻塞,移植腺体失去功能。我们通过家兔的实验研究,明确其病理学基础为导管内唾液滞留及管周炎症细胞浸润,呈现典型慢性阻塞性唾液腺炎症的特点,提出了慢性阻塞性移植下颌下腺炎的疾病概念,并制定了诊断标准:(1)核素检查证实移植腺体成活;(2)腺体反复肿胀,无或少而黏稠的分泌,刺激腺体分泌不增加;(3)改良 Schirmer 试验 < 3 mm;(4)移植腺体造影显示主导管不规则扩张。161 例患者的 174 侧移植腺体的临床分析显示,"休眠期"内发病率为 9.3%,术后远期为 1.3%[32]。

6.2　建立评价移植下颌下腺分泌功能的方法

评价泪液分泌量通常采用 Schirmer 试验,但运动和室温会对移植下颌下腺的分泌产生显著影响,影响检测的准确性。本项目通过 39 侧移植患眼在静息、运动、高温和腺体按摩状态下分泌规律的研究,建立了控制运动和室温等影响的改良 Schirmer 试验技术,提高了对移植下颌下腺分泌功能检测的可信性及准确性[33]。

6.3　建立移植下颌下腺的造影诊断技术

通过移植下颌下腺造影检查研究,我们建立了造影技术规范,明确了正常及阻塞性炎症状态下移植下颌下腺的造影表现,作为慢性阻塞性移植下颌下腺炎重要的客观诊断标准之一[34]。

6.4　建立慢性移植下颌下腺炎的治疗规范

对确诊为慢性阻塞性移植下颌下腺炎的患者,根据病情提出 3 种处理方式进行个体化治疗:(1)强化促分泌:有导管阻塞倾向者,综合使用逆行冲洗、局部热敷、辣椒素涂抹、卡巴胆碱注射,打破低分泌-炎症的恶性循环,预防导管阻塞;(2)导管口重建:阻塞部位位于导管口者,游离后切除阻塞的导管口,剩余导管重新开口于穹窿部结膜;(3)血管移植导管再造:阻塞位于导管中段并有导管高度扩张者,游离后切除阻塞段导管,切取头静脉,进行导管再造[29]。

我们的研究结果提示,精细的设计和手术是保证移植腺体成活和下颌下腺移植获得成功的基础,而个性化有效调控术后移植腺体的分泌是减少并发症及提高患者生活质量的关键。

(志谢:感谢国家科技部、国家自然科学基金委员会、教育部、北京市、原国家卫生部等部门给予的基金支持,感谢项目组全体同事的辛勤工作以及患者和家属的支持与配合。)

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(2017-11-03 收稿)
(本文编辑:任英慧)

A 20-year study on microvascular autologous transplantation of submandibular gland for treatment of severe dry eye

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SUMMARY Severe dry eye is a refractory ophthalmologic disease. Our multidisciplinary research group treated severe dry eye by microvascular autologous transplantation of submandibular gland (SMG) during the past 20 years. The SMG, with its blood vessels and Wharton's duct, was harvested from the submandibular triangle and transferred to the temporal area. The blood vessels in the SMG were anastomosed with the temporal blood vessels using a microsurgical technique. Then, the distal end of Wharton's duct was sutured to form an opening in the upper lateral conjunctival fold. The tear was replaced by the secretion of the transplanted SMG to lubricate the ocular surface. In our study, the surgical techniques of blood vessel management were continuously modified to increase the survival rate of the transplanted SMG. A novel surgical modality of partial transplantation of SMG was established to prevent postoperative epiphora. A clinical study with the largest case number in the world was conducted and the effectiveness of transplantation of SMG for severe dry eye was fully confirmed. In order to resolve two main clinical problems including ductal obstruction resulted from low secretion rate during the latent period, and epiphora due to over secretion of the transplanted SMG in the later term of transplantation, the regulation of the secretion mechanism of the normal and transplanted SMG were investigated. New opinions on mechanisms of saliva secretion were provided. Based on the priniciple of translational medicine, the results of related basic research were applied in the clinic. The clinical guidelines for secretion regulation of transplanted SMG were established. A concept of chronic obstructive sialadenitis of transplanted SMG was provided and its diagnostic criteria, diagnostic technique of sialography, and therapeutic regimen were established. As a result, the surgical success rate was obviously elevated, the surgical complications were decreased, and life quality of the patients was greatly improved.

KEY WORDS Submandibular gland; Dry eye; Keratoconjunctivitis sicca; Transplantation, autologous; Lacrimal apparatus diseases