

## ·实验研究 Experimental research·

## 自制内镜注射针经喉镜注射氢氧化钠构建兔食管良性狭窄模型

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**【摘要】 目的** 探讨一种新的兔食管良性狭窄模型构建方法——自制内镜注射针经喉镜注射氢氧化钠(NaOH)构建食管良性狭窄模型,为支架置入体内相关介入研究提供实验基础。**方法** 自制注射针:将一次性胃镜注射针外层导管去除,取含针头端长约 70 mm 内芯导管,将针头剪至 2 mm 长并磨成斜坡状,去除针柄及部分塑料管,然后接上内芯导管无针头端。选择 2~3 kg 健康兔 10 只,3%戊巴比妥(1 ml/kg)经耳缘静脉注射麻醉,左侧卧位固定于 DSA 操作台;透视下将泥鳅导丝插入胃腔,8 mm×40 mm 球囊置于食管下段贲门处,喉镜下距球囊近端约 1 cm 处选取环周 4 个点,经自制内镜注射针分别注入 1.5% NaOH 0.25 ml。术后 2 周和 4 周分别作喉镜及造影检查;术后 4 周处死实验兔,取材作组织病理学检查。**结果** 术后 2 周喉镜检查显示食管中下段溃疡形成,无明显狭窄;造影显示食管中下段管壁不光整。术后 4 周喉镜显示食管中下段瘢痕形成、食管狭窄;造影显示食管中下段局部狭窄;组织病理学检查提示局部溃疡、黏膜下层增厚、瘢痕形成。食管管径狭窄率为平均 49.54%(44.89%~56.65%);狭窄长度造影测量为平均 18.0 mm(14.6~22.8 mm),标本测量为平均 16.3 mm(13.1~21.1 mm)。**结论** 自制内镜注射针经喉镜注射 1.5% NaOH 可成功构建兔食管良性狭窄模型,方法简单易行,狭窄程度和部位易控。

**【关键词】** 食管狭窄;喉镜;内镜注射针;氢氧化钠;兔模型

中图分类号:R571.1 文献标志码:A 文章编号:1008-794X(2016)-10-0891-05

**The establishment of benign esophageal stricture model in rabbits through laryngoscopic injection of NaOH with a self-made endoscopy injection needle** YANG Kai, LI Xiao-feng, YUAN Tian-wen, ZHOU Bi, ZHU Yue-qi, CAO Jun, CHEN Bin, CHENG Ying-sheng. Department of Radiology, Affiliated Shanghai Sixth People's Hospital, Shanghai Jiaotong University, Shanghai 200233, China

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**【Abstract】 Objective** To discuss a new method for the establishment of benign esophageal stricture in rabbits, i.e. through laryngoscopic injection of sodium hydroxide (NaOH) with a self-made endoscopy injection needle to establish benign esophageal stricture model, in order to provide experimental basis for the interventional studies associated with stent implantation. **Methods** The self-made endoscopic injection needle was prepared as follows. The outer layer of a disposable gastroscopy injection needle was removed in order to obtain an about 70 mm-length inner core catheter with needle tip, this needle tip was cut to 2 mm long and was ground to form a slope shape. The needle handle and part of its plastic tube were cut off, then it was connected with the needle-free end of the inner core catheter. Ten healthy rabbits of 2~3 kg body weight were selected, 3% pentobarbital (1 ml/kg) was injected via ear-margin vein to make general anesthesia. The experimental rabbit was fixed on the DSA operating table in left lateral recumbent position. Under fluoroscopic guidance, the guide wire was inserted into the gastric cavity, an 8 mm by 40 mm balloon was placed at the lower segment of esophagus near the cardia. Under laryngoscopic observation, 4 points around the esophageal lumen, which were about one cm from the proximal end of the balloon, were selected to separately receive the injection of 0.25 ml of 1.5% NaOH through the self-made endoscopic injection needle. Laryngoscope

DOI:10.3969/j.issn.1008-794X.2016.10.014

基金项目:国家自然科学基金(81370041,81371659,81171437,81571773)

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examination and esophagography were performed at 2 and 4 weeks after the injection. The experimental rabbits were sacrificed at 4 weeks after the treatment, and the tissue samples were collected for pathological examination. **Results** Laryngoscope examination at two weeks after operation showed that ulceration was formed at the middle and lower part of the esophagus, but no obvious stricture was observed; esophagography revealed that the mucous membrane of the middle and lower part of esophagus became rough. Laryngoscope examination at four weeks after treatment indicated that there were scar formation and esophageal stenosis at the middle and lower part of the esophagus; esophagography showed that local stenosis was formed at the middle and lower part of the esophagus. Histopathological examination revealed that there were local ulcer, submucosal thickening and scar formation. The mean esophageal stenosis rate was 49.54% (44.89%–56.65%); the mean length of the stricture measured on esophagographs was 18.0 mm (14.6 mm–22.8 mm), which was 16.3 mm (13.1 mm–21.1 mm) when measured on the specimens. **Conclusion** Laryngoscopic injection of 1.5% NaOH with a self-made endoscopy injection needle can successfully establish benign esophageal stricture model in rabbits. This method is simple and easy to operate, besides, the degree and location of the stenosis can be easily controlled. (J Intervent Radiol, 2016, 25: 891-895)

**[Key words]** esophageal stenosis; laryngoscope; endoscopic injection needle; sodium hydroxide; rabbit model

食管良性狭窄为临床常见病症,大多由各种食管和食管-胃手术、早期食管癌内镜下黏膜切除术或黏膜下剥离术、胃食管反流病、误食腐蚀性物质、放射治疗等损伤食管后刺激局部黏膜下纤维结缔组织增生、胶原沉积引起<sup>[1-4]</sup>。文献报道 5%~46%食管癌术后吻合口发生良性瘢痕狭窄<sup>[5-8]</sup>,约占所有食管良性狭窄的 30%<sup>[9]</sup>。进食困难是食管良性狭窄的主要症状,可伴有误吸、反流、体重下降等,严重影响患者生活质量。目前食管良性狭窄动物模型制作方法有氢氧化钠(NaOH)腐蚀法<sup>[10-15]</sup>、光动力法<sup>[16]</sup>、内镜黏膜切除术<sup>[17-18]</sup>和内镜下烧灼法<sup>[19-20]</sup>。NaOH 腐蚀法存在灼伤面积较弥漫、狭窄部位不易控制、易渗入胃内造成胃黏膜损伤等不足,其它方法需要内镜配合、操作复杂。本研究旨在探讨自制注射针喉镜下注射 NaOH 构建兔食管良性狭窄模型的可行性,为食管良性狭窄治疗研究提供一稳定可控的实验模型。

## 1 材料与方法

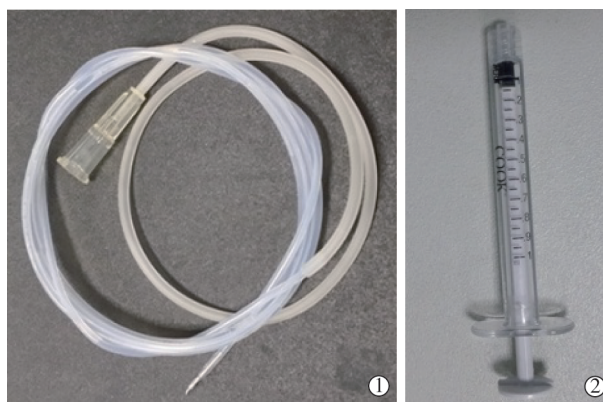
### 1.1 实验动物

健康兔 10 只,雌雄不限,10~11 周龄,体重 2~3 kg,由上海交通大学公共卫生中心实验动物所提供,每只分笼饲养于上海市第六人民医院动物房(相对湿度 40%~50%,温度 22~25℃,光照 12 h 明暗交替),自由饮水摄食。

### 1.2 实验器械及药物

参照文献<sup>[21-23]</sup>报道自制注射针:取一次性胃镜注射针,去除外层导管取含针头端长约 70 mm 内芯

导管,将针头剪至 2 mm 长并磨成斜坡状,使针尖锐利光滑,并用微导丝穿过检查针头是否通畅;去除针柄及部分塑料管,接上内芯导管无针头端(图 1①),注射生理盐水检查导管及针头是否通畅,将其浸泡于消毒液中备用。注射针管为美国 Cook 公司 1 ml 螺口针管(图 1②)。日本 Olympus 公司电子喉镜:长 60 cm,工作通道 2 mm。1.5% NaOH 由上海交通大学实验中心配制,3%戊巴比妥由上海第一生化药业公司生产、上海市第六人民医院动物实验中心配制。



①自制内镜注射针;②Cook 注射针管

图 1 建模用自制注射针图示

### 1.3 建模实验操作

术前兔禁食禁水 24 h,称重;3%戊巴比妥(1 ml/kg)经耳缘静脉注射麻醉,左侧卧位固定于 DSA 操作台;透视下将 2 根普通泥鳅导丝插入胃腔,其中 1 根导丝导引 8 mm×40 mm 球囊置于食管下段贲门处,另 1 根导丝导引 5 F 单弯导管至食管中段,对比

剂经高压泵充盈球囊,注入导管少量,观察有无进入胃腔,撤出 5 F 导管及导丝。

将喉镜送入食管中下段,距离球囊近端约 1 cm 处选取环周 4 个注射点,自制注射针经工作通道于直视下分别注入 1.5% NaOH 0.25 ml,注射持续时间 5 s;注射完毕球囊减压,经注射针注入 20 ml 生理盐水后撤出喉镜及球囊。

术后实验兔禁食 24 h,不禁水,规则喂食;观察进食情况。术后 2 周和 4 周分别作喉镜及造影检查,同时称重。

#### 1.4 组织病理学检查

术后 4 周处死实验兔,开胸分离食管,取出贲门至贲门上方 10 cm 内食管(食管中下段)标本;剖开食管,测量腐蚀处食管壁最大厚度和最窄处管腔周长,计算出最窄处管腔直径,并以此计算食管狭窄指数(食管壁厚度/食管内径);取狭窄处食管组织固定、包埋、切片,作苏木精-伊红(HE)和 Masson 染色<sup>[24-25]</sup>。

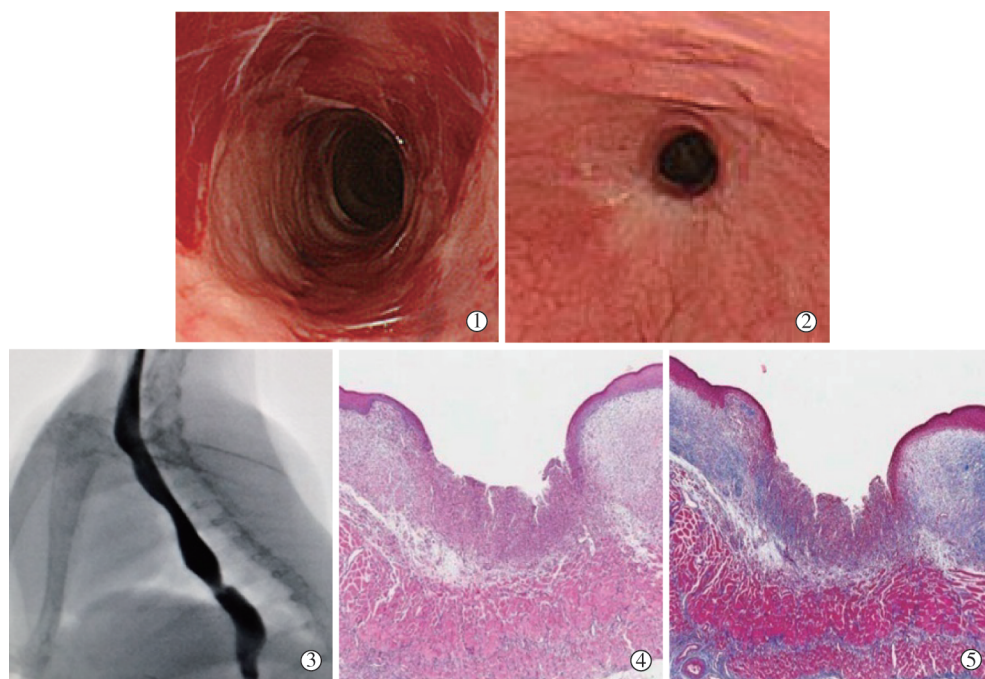
## 2 结果

术后 4 周后实验兔食管管径变化、狭窄情况见表 1。

表 1 术后 4 周后实验兔食管管径变化、狭窄情况

模型	体重/kg		食管直径/mm		狭窄率/%	狭窄长度/mm	
	0 周	4 周	0 周	4 周		造影	标本
1	3.05	2.98	9.7	4.5	46.39	15.9	14.5
2	4.00	3.82	10.4	5.5	52.88	14.6	13.1
3	3.26	3.18	9.8	4.4	44.89	19.2	17.8
4	3.60	3.60	10.3	5.5	56.65	21.4	19.0
5	3.85	3.81	10.3	5.4	52.43	17.6	15.7
6	3.20	3.16	9.9	4.7	47.47	22.8	21.1
7	3.44	3.44	10.0	5.1	51.00	18.9	17.3
8	3.92	3.85	10.1	5.1	50.49	16.7	15.0
9	3.68	3.60	9.8	4.5	45.45	17.5	16.1
10	3.56	3.45	9.7	4.6	47.41	15.4	13.8
平均	3.56	3.23	11.26	5.58	49.54	18.0	16.3

术后 2 周喉镜检查显示食管中下段溃疡形成,无明显狭窄,造影显示食管中下段管壁不光整;术后 4 周喉镜显示食管中下段瘢痕形成、食管狭窄(图 2①②),造影显示食管中下段局部狭窄(图 2③),食管管径狭窄率为 44.89%~56.65%,平均 49.54%,狭窄长度为 14.6~22.8 mm,平均 18.0 mm。组织病理学检查显示,大体标本狭窄段长度为 13.1~21.1 mm,平均 16.3 mm;HE 染色镜下可见小溃疡、黏膜下层增厚、纤维细胞增生(图 2④),Masson 染色镜下可见溃疡、黏膜下层增厚、肌层胶原蛋白沉积(图 2⑤)。



①喉镜检查术后 2 周时食管壁充血、糜烂、溃疡形成;②术后 4 周时食管狭窄;③术后 4 周时食管 DSA 造影显示食管下段狭窄;④组织病理学检查 HE 染色镜下(x200)可见小溃疡、黏膜下层增厚、纤维细胞增生;⑤ Masson 染色镜下(x200)可见溃疡、黏膜下层增厚、肌层胶原蛋白沉积

图 2 术后喉镜、DSA、病理检查结果

## 3 讨论

食管良性狭窄指除肿瘤以外其它原因引起的

狭窄,临床常见的主要有:①各种食管疾病及食管-胃手术后吻合口瘢痕狭窄;②最近开展的早期食管



癌内镜下黏膜切除、黏膜剥离术后瘢痕狭窄;③胃食管反流病、腐蚀性物质、放射治疗等损伤食管后黏膜下纤维结缔组织增生、胶原沉积所致瘢痕狭窄<sup>[1-4]</sup>。微创治疗目前已逐渐取代外科手术成为食管良性狭窄性病变的主要治疗方法,临床上常用支架置入术,但永久性支架易发生移位、再狭窄及出血等并发症,因而也是临床研究热点。构建动物模型是研究食管良性狭窄必不可少有效前提,我们通过自制内镜注射针经喉镜注射 NaOH 探讨食管良性狭窄模型构建新方法,为支架置入体内相关研究提供实验基础。

医学动物实验选择原则为近似性、易化性、相容性和可获得性<sup>[26]</sup>。目前食管良性狭窄模型制作常用实验动物有大鼠、猫、兔、犬和猪等。我们依据上述原则选择兔构建动物模型,其优点:①食管肌层有 3 层(内、外纵肌层,中间环肌层),该结构耐腐蚀、不易穿孔<sup>[11]</sup>;②食管可予喉镜检查,利于模型制作;③饲养简单方便、经济。

目前构建食管良性狭窄动物模型的方法包括 NaOH 腐蚀法<sup>[10-15]</sup>、光动力法<sup>[16]</sup>、内镜黏膜切除或黏膜下剥离法<sup>[17-18]</sup>、内镜下烧灼法<sup>[19-20]</sup>。NaOH 腐蚀法通过各种方法、不同浓度 NaOH 溶液腐蚀食管中下段,构建食管瘢痕狭窄,但存有不足:①20 多年前主要通过开胸制作,创伤大、死亡率高、狭窄干扰因素多;②近 10 余年多采用内镜下或 DSA 下食管下段注入法,前者因常规食管镜或胃镜镜体较粗,需要超细内镜,成本大,后者 DSA 下操作不够精细;③NaOH 溶液易反流,引起呼吸道损伤或流入胃内,导致胃损伤或穿孔,死亡率高,可重复性差;④腐蚀范围不可控,损伤范围大,狭窄部位不易控。Perry 等<sup>[16]</sup>采用光动力法诱导猪食管良性狭窄模型,方法简单、可重复性强,但需要光敏剂、激光源及光传导纤维,对实验设备要求高。内镜黏膜切除或黏膜下剥离法构建食管良性狭窄模型可行,需要超细内镜支持,全周黏膜切除或黏膜下剥离导致食管狭窄时间较长,并有手术相关出血和穿孔等并发症。内镜下烧灼法需要超细内镜,费用及要求高。

无论是光动力法、内镜黏膜切除或黏膜下剥离法还是烧灼法,均需要在超细内镜下完成,对实验器械及操作技巧要求较高。由于不具备相关条件,本研究未予选择。鉴于上述 NaOH 腐蚀法不足,我们在本研究中设计内镜下黏膜下注射 NaOH 新方法构建兔食管良性狭窄模型。由于支气管镜、食管

镜、膀胱镜均为不锈钢镜筒,质太硬、视野不好、视野下注射困难,我们选择电子喉镜,注射针管为 Cook 公司 1 ml 螺口针管(能精确定量、注射时溶液不会渗漏);实验中发现导管末端通过加热插入针头简单可行,但针头不能很好固定、会缩回,故选择一次性胃镜注射针作改制<sup>[21-23]</sup>;胃镜注射针外层导管直径>2 mm,不能通过电子喉镜钳道,去除外层塑料导管并取含针头端长约 70 mm 内芯导管(电子喉镜长 60 cm),将针头剪至 2 mm 长,然后将其磨成斜坡状,针尖锐利光滑,用微导丝穿过检查针头是否通畅,再将头皮针柄及部分塑料管去掉,接上内芯导管无针头端。本研究自制注射针并通过喉镜下注射 NaOH 溶液腐蚀食管中下段构建食管良性狭窄模型,其优点:①喉镜直径为 6 mm,可替代直径 5 mm 超细内镜;②喉镜直视下注射 NaOH 溶液,腐蚀部位和范围可控,不仅可避免呼吸道损伤,而且防止远段食管及胃壁损伤。缺点是喉镜无吹气功能,不能扩张远段食管、视野不够开阔。我们使用球囊,既可扩张远段食管、开阔视野,同时阻滞 NaOH 溶液,保护远段食管及胃壁免受损伤。

腐蚀法对 NaOH 浓度和方法有一定要求,并发症发生率差异较大。Thompson<sup>[11]</sup>采用 1.0 ml 4% NaOH 构建兔食管狭窄模型,结果有 4 只兔出现呼吸并发症死亡,1 只兔腹部内脏穿孔致死。Turkyilmaz 等<sup>[13]</sup>用缝合线结扎大鼠远段食管长约 2 cm,24 F 导管置入至隔离段食管,经导管滴注 10%NaOH 腐蚀黏膜,避免呼吸损伤。Hwang 等<sup>[15]</sup>采用球囊封堵食管下段,防止腐蚀剂腐蚀远段食管壁和胃壁,超细内镜下经 5 F 导管注入 1.5% NaOH 1 ml 至食管中下段腐蚀黏膜,结果显示腐蚀范围可控,并发症少。综合相关文献报道<sup>[10-15]</sup>,我们选择 1.5% NaOH 溶液作为本研究所用腐蚀剂:①浓度过大(>3%)易导致穿孔,损伤远段食管及胃壁黏膜,甚至腐蚀邻近气管;②由于采用经针注射法,溶液作用部位为黏膜下层或固有肌层,小浓度缓慢作用(注射时间为 5 s/注射点)于局部,腐蚀持续时间长、效果好;③小浓度腐蚀剂对远段食管损伤作用小、并发症少。

本研究结果显示,10 只兔食管良性狭窄模型均构建成功,无并发症发生,食管狭窄率为 49.54%,狭窄长度为 18 mm;表明自制内镜注射针经喉镜注射 1.5% NaOH 溶液腐蚀食管安全、有效,狭窄程度、范围和部位易控,值得推广。

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(收稿日期:2016-02-03)

(本文编辑:边 皓)