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等量幽门螺杆菌不同灌胃方法对小鼠胃黏膜的影响

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[摘要] 目的：探讨等量幽门螺杆菌不同灌胃方法对小鼠幽门螺杆菌的感染率及小鼠胃黏膜感染幽门螺杆菌程度的影响。**方法：** 将含等量NCTC11637幽门螺杆菌的布氏肉汤混悬液通过A，B，C，D 4种方法感染6周龄的雄性C57BL/6小鼠。A方法用幽门螺杆菌悬液0.2 mL/只灌胃小鼠，隔天1次，共5次。B方法用幽门螺杆菌悬液0.2 mL/只灌胃小鼠，每天1次，连续5次。C方法第1天用幽门螺杆菌悬液灌胃小鼠0.4 mL/只，以后连续3 d每天灌胃1次，每次0.2 mL/只。D方法第1天用幽门螺杆菌悬液灌胃小鼠0.4 mL/只，以后隔天灌胃1次，每次0.2 mL/只，共3次。E方法用等量生理盐水灌胃小鼠。灌胃后第2，4，6周处死小鼠，其胃黏膜组织用快速尿素酶检测是否有幽门螺杆菌感染，并取胃黏膜组织进行HE染色，观察胃黏膜组织的感染程度。**结果：**2周后A，B，C，D组小鼠的感染率分别为33.3%，50.0%，66.7%，33.3%；炎症的感染程度C方法>B方法>D方法>A方法>E方法。4周后A，B，C，D组小鼠的感染率分别为50.0%，83.3%，83.3%，66.7%；炎症的感染程度C方法>B方法>D方法>A方法>E方法。6周后A，B，C，D组小鼠的感染率均为100%；炎症的感染程度C方法>D方法>B方法>A方法>E方法。**结论：**在幽门螺杆菌急性感染期，灌胃方法不同小鼠的感染率不同，炎症程度不同；在幽门螺杆菌的慢性感染期，灌胃方法不同小鼠的感染率相同，感染的炎症程度不同。采用第1天灌0.4 mL，以后连续3 d每天1次，0.2 mL/次的灌胃方法最有利于幽门螺杆菌在小鼠胃黏膜定植，感染炎症程度最严重，有利于幽门螺杆菌感染模型的成功建立。

[关键词] 等量幽门螺杆菌悬液；灌胃方法；胃黏膜；小鼠

Effect of equal concentration of *Helicobacter pylori* suspension on gastric mucosa in mice by different gavage methods

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ABSTRACT

Objective: To investigate the effects of equal concentration of *Helicobacter pylori* suspension on gastric mucosal infection in mice by different gavage methods.

Methods: Six-week-old male C57BL/6 mice were infected by a suspension of Brucella broth containing the same amount of NCTC11637 *Helicobacter pylori* suspension by A, B, C, and D methods. For method A, the mice were intragastrically administered with *Helicobacter*

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pylori suspension (0.2 mL per mouse), once two day for 5 times; for method B, the mice were intragastrically administered with *Helicobacter pylori* (0.2 mL per mouse) once a day for 5 times; for method C, the mice were perfused with 0.4 mL per mouse of *Helicobacter pylori* suspension on the first day, then once a day and 0.2 mL per mouse for 3 times; for method D, the mice were administrated with 0.4 mL per mouse *Helicobacter pylori* suspension on the first day, 0.2 mL per mouse every other day for 3 times. For method E, the mice received equal amounts of normal saline. The mice were killed at 2, 4, and 6 weeks after gavage. The gastric mucosa was detected by rapid urease test for *Helicobacter pylori* infection, and gastric mucosa was taken for HE staining to observe the degree of infection.

Results: After 2 weeks of gavage, the infection rates of the mice in A, B, C, and D group were 33.3%, 50.0%, 66.7%, and 33.3%, respectively. The degree of inflammation infection was as following order: C group>B group>D group>A group>E group. The infection rates of mice after 4 weeks of gavage in the A, B, C, and D groups were 50.0%, 83.3%, 83.3%, and 66.7%, respectively. The degree of inflammation infection was as following order: C group>B group>D group>A group>E group. After 6 weeks of gavage, the infection rate in A, B, C, and D groups was 100%, while the degree of inflammation infection was as following order: C group>D group>B group>A group>E group.

Conclusion: At the acute stage of *Helicobacter pylori* infection, different gavage methods show different infection rates in mice, and the degree of inflammation is different. At the chronic stage, different gavage methods display the same infection rate in mice with different degree. The gavage method that 0.4 mL *Helicobacter pylori* suspension on the first day, then once a day and 0.2 mL for 3 times is most conducive to *Helicobacter pylori* colonization in the gastric mucosa of mice. This method can induce the the most seriou inflammatory infection and is beneficial to the successful establishment of the *Helicobacter pylori* infection model.

KEY WORDS equal amount of *Helicobacter pylori* suspension; different gavage methods; gastric mucosa; mice

幽门螺杆菌(*Helicobacter pylori*，*Hp*)是一种广泛定植于胃黏膜的革兰氏阴性菌，是厌氧性的螺旋形细菌[1]，是目前在胃里发现的唯一的微生物。全球大约有一半以上的人口感染*Hp*，不同地区*Hp*的感染率不同，非洲最高为70.1%，拉丁美洲和加勒比海地区为63.4%，亚洲地区也高达54.7%[2-4]。中国农村人口*Hp* 感染率为 66%，城市人口则为 47%(1983年至2013年)[5-6]。2017年，*Hp*感染[7]被世界卫生组织国际癌症研究机构列入了致癌物清单。*Hp*的长期感染可以导致胃黏膜萎缩和肠化生[8-9]，与多种消化系统疾病相关，如慢性胃炎、消化性溃疡、胃癌、胃黏膜相关淋巴组织淋巴瘤(胃MALT淋巴瘤)等[10-11]。其可以通过多种毒力因子损害人类胃上皮细胞并逃避宿主的免疫反应以造成慢性持续性感染。为了研究*Hp*的致病机制及毒力因子，出现了不同的造模方法。在建立动物模型时，*Hp*菌株的选择对造模至关重要[12]，目前常用的*Hp*有SS1，NCTC11637，猫胃螺杆菌等，其中NCTC11637是公认的标准致病菌[13]。造模小鼠的主要类型有BALB-C小鼠、C57BL/6小鼠、昆明小鼠、蒙古沙鼠等不同类型[14]。研究[15-16]证明不同致病性*Hp*对同一品系小鼠感染率不同，同一*Hp*对不同品系小鼠感染率有所差异。但至今鲜有文献报道同一种*Hp*用不同的灌胃方法，感染同一品系小鼠的感染率是否有差异。因此本研究主要通过不同的灌胃方法建立*Hp*感染模型，通过检测*Hp*的感染率及HE染色观察胃炎程度，旨在比较不同灌胃方法对小鼠*Hp*感染率及胃黏膜感染程度的影响。

1 材料与方法

1.1 材料

C57BL/6雄性小鼠90只，6~7周龄，体重18~20 g，购自重庆腾鑫实验动物公司；*Hp*是西南医科大学附属医院消化科*Hp*菌库中冻存的NCTC11637(第三军医大学惠赠)。

1.2 方法

1.2.1 细菌的复苏、传代及鉴定

从西南医科大学附属医院消化科*Hp*菌库–80 ℃冰箱中取出 NCTC11637($CagA^+$，$VacA^+$)标准*Hp*菌株，于液体培养基(布氏肉汤) 进行复苏，将装有*Hp*菌株的液体培养基置于在37 ℃，5%O_2，10%CO_2，85%N_2的三气培养箱中复苏、生长。2 d后取少量悬液放入快速尿素酶液体中鉴定培养的细菌，鉴定为*Hp*后进行传代培养，传2代后将经鉴定的*Hp*用分光度仪检测光密度(opitical density，OD)值，将OD值调整为1的悬

液(OD=1，细菌的数量达1×10^9 CFU/mL)，置摇床上震荡培养20 h。

1.2.2 分组

90只6周龄C57BL/6 雄性小鼠，适应性用普通饲料喂养1周，然后分为A，B，C，D，E组，每组18只小鼠。

第1次灌胃前1天将5组小鼠用三联疗法(甲硝唑2.6 mg/只，丽珠得乐1.2 mg/只，阿莫西林5 mg/只)清除小鼠胃内杂菌，禁食禁饮12 h。灌胃前1 h给予2%碳酸氢钠0.2 mL/只灌胃，然后分别用5种方法处理。A方法：用 OD=1的NCTC11637 *Hp*悬液0.2 mL/只灌胃小鼠，隔天灌1次，共5次。B方法：用 OD=1的NCTC11637*Hp* 悬液0.2 mL/只灌胃小鼠，每天1次，连续5次。C方法：用OD=1的NCTC11637 *Hp*悬液灌胃小鼠，第1天灌0.4 mL/只，以后连续3 d每天灌胃1次，每次0.2 mL/只。D方法：用OD=1的NCTC11637 *Hp*悬液灌胃小鼠，第1天灌0.4 mL/只，以后隔天灌胃1次，每次0.2 mL/只，共3次。E方法：小鼠用生理盐水灌胃。

10 d后小鼠灌胃完成，然后于第2，4 ，6周分别禁食24 h，每组处死6只小鼠，使用无菌手术器械取出胃组织，以PBS冲洗胃部残渣，将胃腺部剪下，一份置于快速尿素酶试剂内。一份用多聚甲醛固定，石蜡切片，HE 染色后在倒置显微镜下观察胃炎程度，确定 *Hp*的感染程度。

1.2.3 胃黏膜病理改变标准确定

慢性胃炎病理(显微镜下)观察5项组织学变化：1)*Hp*感染；2) 慢性炎性反应(单个核细胞浸润)；3)活动性(中性粒细胞浸润)；4)萎缩(固有腺体减少)；5)肠化(肠上皮化生)。炎症的分级：0提示无，+提示轻度，++提示中度，+++提示重度。

1.2.4 快速尿素酶检测

将小鼠胃腺部剪下，剪成细碎组织，将组织置于快速尿素酶试剂中，若快速尿素酶由黄变红则*Hp*感染阳性，反之则阴性。

1.3 统计学处理

采用SPSS 19.0统计软件进行数据分析，各组间比较用Fisher精确概率法检验，P<0.05为差异有统计学意义。

2 结 果

2.1 用不同方法灌胃等量*Hp*悬液结束后第2，4及6周小鼠胃黏膜*Hp*感染率

灌胃结束后第2周小鼠的*Hp*感染率C组最高，但5组间比较差异无统计学意义(P>0.05)；第4周小鼠的*Hp*感染率在B组和C组中最高，5组间比较差异有统计学意义(P<0.05)；第6周小鼠的*Hp*感染率均为100%，E组*Hp*感染率为0，5组间比较差异有统计学意义(P<0.001，表1)。

2.2 用不同方法灌胃等量*Hp*悬液结束后第2，4及6周小鼠胃黏膜炎症程度比较

用不同方法灌胃结束后第2周小鼠胃黏膜炎症程度C组>B组>D组>A组>E组；4周后小鼠胃黏膜炎症程度C组>B组>D组>A组>E组；6周后小鼠胃黏膜炎症程度C组>D组>B组>A组>E组(表2，图1~3)。

表1 用不同方法灌胃等量*Hp*悬液结束后第2，4及6周小鼠胃黏膜*Hp*的感染率

Table 1 Infection rate of gastric mucosa *Hp* in mice at the 2nd, 4th, and 6th weeks after administered with the same amount of *Hp* suspension by different gavage methods

周数	*Hp*感染率/%					*P*	Fisher值
	A组	B组	C组	D组	E组		
2	33.3	50.0	66.7	33.3	0	0.225	6.323
4	50.0	83.3	83.3	66.7	0	0.025	11.335
6	100.0	100.0	100.0	100.0	0	<0.001	21.01

表2 用不同方法灌胃等量*Hp*悬液后在不同时间小鼠胃炎的分级

Table 2 Grading for gastritis after administered with the same amount of *Hp* suspension by different gavage methods at different time

周数	胃炎分级				
	A组	B组	C组	D组	E组
2	0	++	+++	+	0
4	+	++	++++	+++	0
6	++	+++	+++++	++++	0

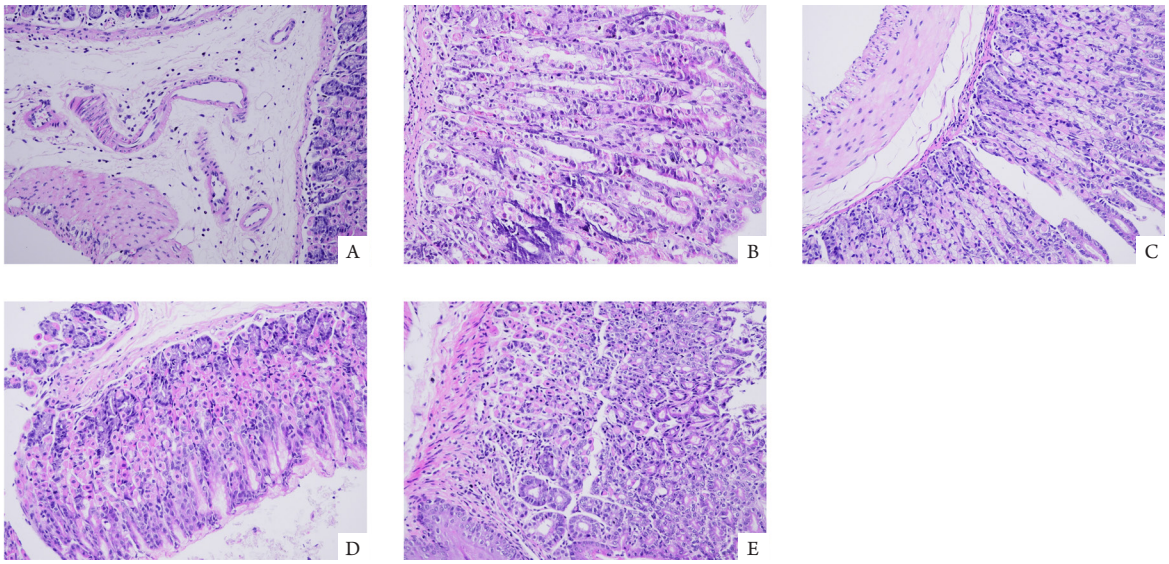

图1　用不同方法灌胃等量*Hp*悬液结束后第2周小鼠胃黏膜炎症改变(HE，×400)

Figure 1　Changes in gastric mucosal inflammation at the 2nd week after administered with the same amount of *Hp* suspension by different gavage methods (HE, ×400)

A: A method; B: B method; C: C method; D: D method; E: E method

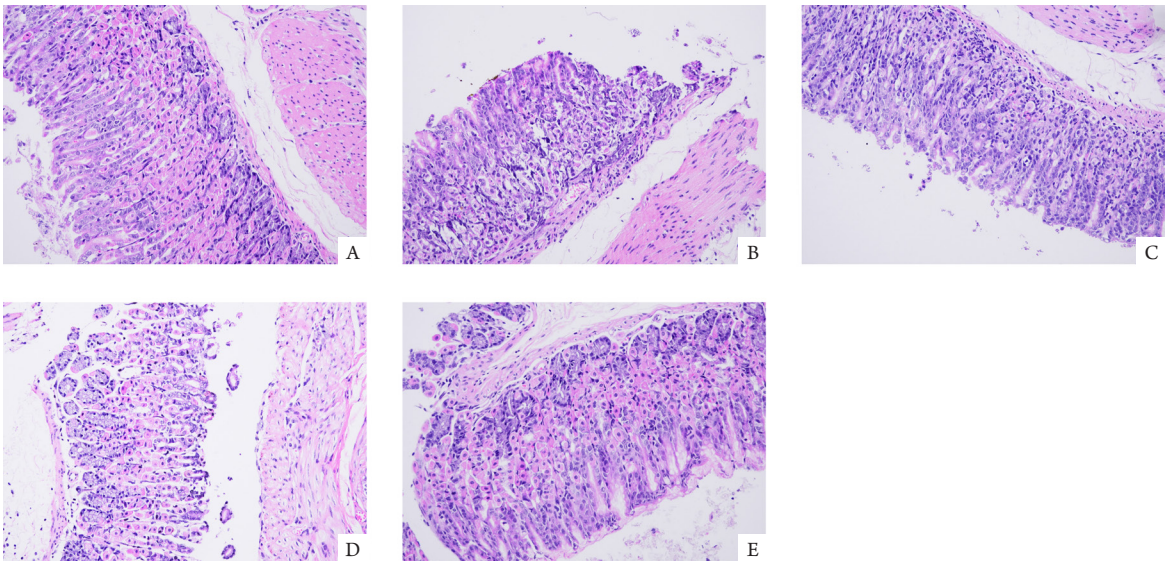

图2　用不同方法灌胃等量*Hp*悬液结束后第4周小鼠胃黏膜炎症改变(HE，×400)

Figure 2　Changes in gastric mucosal inflammation at the 4th week after administered with the same amount of *Hp* suspension by different gavage methods (HE, ×400)

A: A method; B: B method; C: C method; D: D method; E: E method

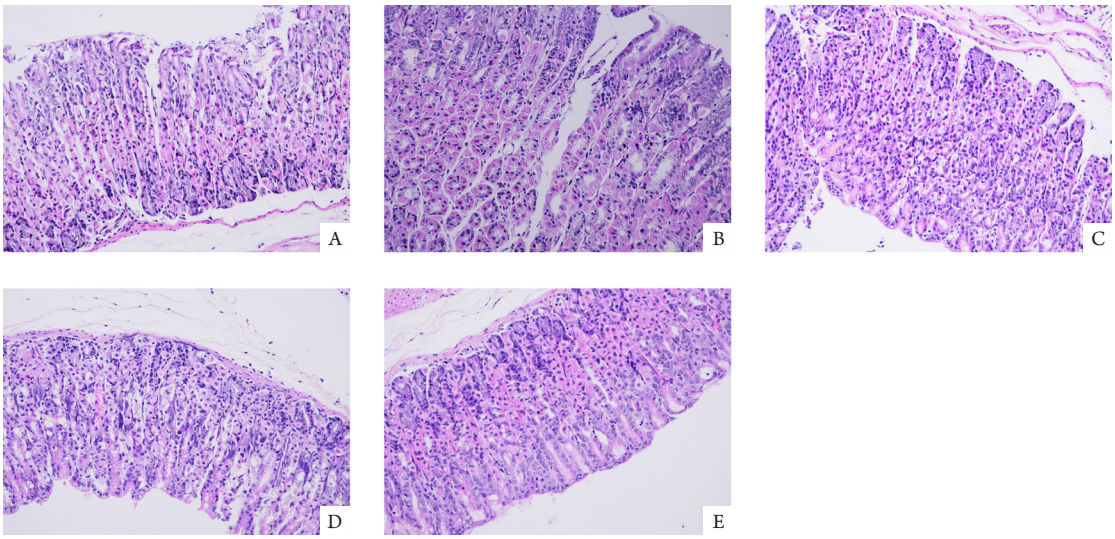

图3 用不同方法灌胃等量*Hp*悬液结束后第6周小鼠胃黏膜炎症改变(HE，×400)

Figure 3 Changes in gastric mucosal inflammation at the 6th week after administered with the same amount of *Hp* suspension by different gavage methods (HE, ×400)

A: A method; B: B method; C: C method; D: D method; E: E method

3 讨 论

建立小鼠*Hp*感染模型的方法多种多样，连大卫等[17]用SS1菌株感染C57BL/6建立*Hp*感染的模型；张荣光等[18]用NCTC11637菌株感染昆明小鼠建立*Hp*感染的模型；周曾芬等[19]用CagA阳性、VacA阳性的*Hp*感染BALB/c小鼠。在不同的研究中针对不同目的采用不同的造模方法，本研究通过将等量的NCTC11637菌株用不同的灌胃方法感染C57BL/6小鼠，比较小鼠胃黏膜*Hp*的感染率及炎症程度，结果显示小鼠用4种方法灌胃结束后2周各组小鼠之间*Hp*感染率差异无统计学意义；但是灌胃结束后4周和6周各组小鼠之间*Hp*感染率差异有统计学意义，表明不同灌胃方法对小鼠*Hp*急性感染期的感染率没有影响；但是对小鼠*Hp*慢性感染期的感染率有影响，其中C方法小鼠*Hp*感染率最高，因此在建立小鼠*Hp*慢性感染模型时，笔者推荐采用C方法，此方法最利于提高小鼠*Hp*感染率。就小鼠胃黏膜炎症程度而言，灌胃结束后2，4，6周各组小鼠的胃黏膜随着时间延长，炎症程度逐渐加重；当感染时间相同时，C方法感染的小鼠胃黏膜炎症最重。综上，无论*Hp*感染率还是小鼠胃黏膜炎症程度，C方法更优，这为以后有效建立*Hp*感染的模型提供了依据。

本实验研究虽然表明C方法更有利于*Hp*感染模型的建立，但尚未对不同灌胃方法造成小鼠*Hp*感染率及感染炎症程度不同的机制进行进一步探究。已有研究[20]报道*Hp*有5~6根鞭毛，使其能够穿过胃黏膜表面的黏液层而在胃黏膜上皮细胞表面定植。当*Hp*定植于胃黏膜表面时会使此处的胃酸分泌增加，更有利于其他新入侵的*Hp*进一步定植。1993年Boren等[21]首先报道*Hp*与胃黏膜上皮细胞的结合依赖于Lewis b抗原的表达，*Hp*生物被膜的形成是参与定植的重要因素；Warren等[23]通过诱导小鼠胃部病变，观察到*Hp*可以迅速识别小鼠胃黏膜损伤部位，同时定植，在短短几分钟之内，累积的细菌可以干扰组织的修复。因此，笔者推测当*Hp*对定植处组织造成损伤后，短时间内如有新入侵的*Hp*，可以快速识别胃黏膜损伤部位，累积的细菌入侵造成组织损伤进一步加重，既有利于细菌再次定植，也使胃组织感染进一步加重。但是其具体机制仍有待于进一步探索。

利益冲突声明：作者声称无任何利益冲突。

参考文献

[1] 梁露, 曾文明. 胃黏膜相关淋巴组织(MALT)淋巴瘤的病理诊断[J]. 当代医学, 2016, 22(7): 26-27.
LIANG Lu, ZENG Wenming. Pathological diagnosis of gastric mucosa-associated lymphoid tissue lymphoma[J]. Contemporary Medicine, 2016, 22(7): 26-27.

[2] 唐鄂. 上海市青浦区干部体检幽门螺杆菌情况调查[J]. 临床临床误诊误治, 2009, 22(12): 5-7.
TANG E. The investigation to the *Hp* infection condition of the cadres in Qingpu District Shanghai City[J]. Clinical Misdiagnosis & Mistherapy, 2009, 22(12): 5-7.

[3] 张玫, 汤哲, 周英智, 等. 北京地区老年人群幽门螺旋杆菌感染的血清流行病学研究[J]. 中华流行病学杂志, 2005, 26(9): 687-689.
ZHANG Mei, TANG Zhe, ZHOU Yingzhi, et al. Seroepidemiological study on *Helicobacter pylori* (CagA, VacA) infection of elderly people in Beijing[J]. Chinese Journal of Epidemiology, 2005, 26(9): 687-689.

[4] 陈月梅, 王敏, 张婷婷, 等. 8 536例健康体检者幽门螺旋杆菌血清尿素酶抗体阳性率分析[J]. 实用预防医学, 2012, 19(2): 254-255.
CHEN Yuemei, WANG Min, ZHANG Tingting, et al. Analysis on sero-prevalence of ureas antibody in *Helicobacter pylori* among 8 853 healthy examinees[J]. Practical Preventive Medicine, 2012, 19(2): 254-255.

[5] Handa O, Naito Y, Yoshikawa T. *Helicobacter pylori*: a ROS-inducing bacterial species in the stomach[J]. Inflamm Res, 2010, 59(12): 997-1003.

[6] 赵迪, 王素娟, 张静, 等. 新乡地区儿童幽门螺杆菌流行病学调查分析[J]. 新乡医学院学报, 2013, 30(3): 193-194.
ZHAO Di, WANG Sujuan, ZHANG Jing, et al. Epidemiological investigation of *Helicobacter pylori* infection in children in Xinxiang areas[J]. Journal of Xinxiang Medical College, 2013, 30(3): 193-194.

[7] 赵红珍, 王盖昊, 王兆林, 等. 幽门螺杆菌感染与胃肠道疾病的研究进展[J]. 解放军医药杂志, 2016, 28(1): 110-114.
ZHAO Hongzhen, WANG Gaihao, WANG Zhaolin, et al. Progress in *Helicobacter pylori* infection and gastrointestinal diseases[J]. Medical & Parmaceutical Journal of Chinese People's Liberation Army, 2016, 28(1): 110-114.

[8] 李慕然, 刘艳迪, 唐涛, 等. 幽门螺杆菌和慢性胃炎和慢性胃炎胃黏膜病理变化的关系研究[J]. 天津医药, 2015, 43(1): 54-56.
LI Muran, LIU Yandi, TANG Tao, et al. Study on the relationship between the pathological change of chronic gastritis and *Helicobacter pylori*[J]. Tianjin Medical Journal, 2015, 43(1): 54-56.

[9] Park YH, Kim N. Review of atrophic gastric and intestinal metaplasia as a premalignant lesion of gastric cancer[J]. Cancer Prev, 2015, 20(1): 25-40.

[10] Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylri* infection[J]. Clin Microbiol Rev, 2006, 19(3): 449-490.

[11] Vogiatzi P, Cassone M, Luzzi I, et al. *Helicobacter pylri* as a class I carcinogen: physiopathology and management strategies[J]. J Cell Biochem, 2007, 102(2): 264-273.

[12] Smythies LE, Chen JA, Lindsey JR, et al. Quantitative analysis of *Helicobacter pylori* infection in a mouse model[J]. Immunol Methods, 2000, 242: 67-78.

[13] Xiang Z, Censini S, Bayeli PF, et al. Analysis of expression of CagA and VacA virulence factors in 43 strains of *Helicobacter pylori* reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin[J]. Infect Immun, 1995, 63(1): 94-98.

[14] Go MF, Crowe SE. Virulence and pathogenicity of *Helicobacteri pylori*[J]. Gastroenterol Clin North Am, 2000, 29(3): 649-670.

[15] Aydin O, Egilmez R, Karabacak T, et al. Interobserver variation in histopathological assessment of *Helicobacter pylori* gastritis[J]. World J Gastroenterol, 2003, 9(10): 2232-2235.

[16] Murakami K, Kodama M, Fujioka T. Latest insights into the effects of *Helicabacter pylori* infection on gastric carcinogenesis[J]. World Gastroenterol, 2006, 12(8): 2713-2720.

[17] 连大卫, 扶丽君, 许艺飞, 等. 幽门螺杆菌感染小鼠慢性胃炎模型的建立及评价[J].中国比较医学杂志, 2017, 27(7): 6-12.
LIAN Dawei, FU Lijun, XU Yifei, et al. Establishment and evaluation of chronic gastritis model in mice infected with *Helicobacter pylori*[J]. Chinese Journal of Comparative Medicine, 2017, 27(7): 6-12.

[18] 张荣光, 段广才, 范清堂, 等. 幽门螺杆菌感染的昆明小鼠模型的建立[J]. 中华医学杂志, 2006, 86(12): 857-858.
ZHANG Rongguang, DUAN Guangcai, FAN Qingtang, et al. Establishment of Kunming mouse model of *Helicobacter pylori* infection[J]. National Medical Journal of China, 2006, 86(12): 857-858.

[19] 罗萍, 周曾芬. 建立幽门螺杆菌感染BALB/c小鼠动物模型[J]. 实用临床医学杂志, 2013, 1(27): 3-6.
LUO Ping, ZHOU Zengfen. The establisment of BALB/c mice animal model with *Helicobacter pylori* infection[J]. Journal of Clinical Medicine in Practice, 2013, 1(27): 3-6.

[20] Forstneric V, lvicak-Kocjan K, Plaper T, et al. The role of the C-terminal DO domain of flagellin in activation of Toll like receptor 5[J]. PLoS Pathog, 2017, 13: e1006574.

[21] Borén T, Falk P, Roth KA, et al. Attachment of *H. Pylori* to human gastric epithelium mediated by blood group antigens[J]. Science, 1993, 262(5141): 1892-1895.

[22] Coticchia JM, Sugawa C, Tram VR, et al. Presence and density of *Helicobacter pylori* biofilms in human gastric mucosa in patients with peptic ulcer disease[J]. J Gastrointest Surg, 2006, 10(7): 883-889.

[23] Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis[J]. Lancet, 1983, 1(8336): 1273-1275.

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