

激光照射足三里穴对慢性萎缩性胃炎大鼠胃酸分泌及 NO 含量的影响

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【摘要】 目的 探讨氦氖激光照射足三里穴对慢性萎缩性胃炎(CAG)大鼠胃液 pH 值、胃黏膜壁细胞超微结构及血清中一氧化氮(NO)含量的影响。**方法** 采用随机数字表法将 60 只健康雄性 Wistar 大鼠分成正常对照组、模型组、药物组、针刺组及激光穴位组。通过给予水杨酸钠、酒精灌胃并结合禁食禁水、跑步劳累等干预将模型组、药物组、针刺组及激光穴位组大鼠制成 CAG 动物模型。确认造模成功后,药物组、针刺组及激光穴位组大鼠分别给予药物(如延参健胃胶囊、康复新液灌注)、针刺、激光穴位照射干预。于治疗 14 d 后断头取血,采集稀释胃液及部分胃组织;利用硝酸还原酶法检测血清中 NO 含量,采用酸度计测定胃液 pH 值,通过透射电镜观察胃黏膜超微结构变化。**结果** 模型组大鼠胃液 pH 值、血清中 NO 含量均较正常对照组明显升高($P<0.05$),其壁细胞胞膜结构缺失、部分溶解,线粒体肿胀。药物、针刺足三里穴及激光照射足三里穴均能降低胃液 pH 值及血清中 NO 含量,并且以激光穴位组胃液 pH 值及血清中 NO 含量的改善情况尤为显著,与模型组间差异具有统计学意义($P<0.05$),其壁细胞胞膜结构完整,胞浆细胞器基本恢复正常。**结论** 激光照射足三里穴能促进 CAG 大鼠壁细胞结构及功能恢复,提高胃酸分泌并降低血清 NO 水平,对缓解 CAG 病情具有较好疗效,该疗法值得临床进一步研究、推广。

【关键词】 激光穴位照射; 慢性萎缩性胃炎; 一氧化氮; 超微结构

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Effects of acupoint laser irradiation on gastric acid secretion and NO content in chronic atrophic gastritis

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【Abstract】 Objective To study the effects of He-Ne laser irradiation on the pH value of gastric juice, the ultrastructure of gastric parietal cells and the content of nitric oxide (NO) in chronic atrophic gastritis (CAG). **Methods** Sixty healthy male Wistar rats were randomly divided into a normal control group, a model group, a drug group, an acupuncture group and a laser acupoint group, each of 12. A CAG model was successfully introduced to all except those in the normal control group through the administration of sodium salicylate and alcohol, combined with fasting and water deprivation, running fatigue and other interventions. The rats of the drug, acupuncture and laser acupuncture groups were given medications, acupuncture at Zusanli acupoint and laser irradiation at Zusanli acupoint accordingly. After 14 days of treatment, all of the rats were executed and diluted gastric juice and gastric tissues were collected. NO content in serum was determined using a nitrate enzyme reduction method, the pH value of gastric juice was measured, and the ultrastructure of the gastric mucosa was observed using a transmission electron microscope. **Results** The average pH of the gastric juice and the average serum NO content were both significantly higher in the model group than in the normal control group. In the model group the membrane structure of the parietal cells was absent or partly dissolved and the mitochondria were swollen. Drugs, acupuncture and laser irradiating at the Zusanli acupoint all corresponded with reduced pH and NO content, but the most significant improvements were observed in the laser acupuncture group. In the laser acupuncture group the membrane structure of parietal cells was complete, and the cytoplasmic organelles had nearly returned to normal. **Conclusions** Laser irradiation can promote recovery from CAG, increasing the secretion of gastric acid and de-

creasing the serum level of NO. Both have a good curative effect for CAG. Laser irradiation deserves further clinical research and promotion.

【Key words】 Laser acupuncture; Lasers; Acupuncture; Gastritis; Nitric oxide

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慢性萎缩性胃炎 (chronic atrophic gastritis, CAG) 是一种慢性炎性病变, 患者主要病理特征为胃黏膜发生腺体萎缩性改变, 是我国消化系统领域中的常见病及疑难病, 其发病率居各种胃病之首, 约 5%~10% 的患者可进一步发生癌变^[1-2]。目前临床针对 CAG 患者尚无完善、有效的治疗方案^[3]。中医认为足三里为胃经合穴, 针灸足三里穴能发挥疏通经络、调和气血、健脾健胃功效, 对胃部疾患具有治疗作用^[4]。已有研究表明, 氦氖激光穴位照射对炎症性疾病具有较好治疗作用^[5-6]。为探索 CAG 的科学治疗方案, 本课题组在成功制备 CAG 动物模型后, 分别采用药物 (如延参健胃胶囊、康复新液灌注)、传统针刺及氦氖激光照射足三里穴对 CAG 大鼠进行干预, 并通过检测大鼠胃液 pH 值、胃黏膜超微结构及血清中 NO 含量变化, 分析激光穴位照射治疗 CAG 的疗效, 为临床治疗 CAG 患者提供参考资料。

材料与方法

一、实验动物

共选取清洁级健康 Wistar 大鼠 60 只 [动物合格证号 SCXK (京) 2010-0006], 由北京首都医科大学实验动物部提供, 大鼠体重 180~220 g, 雄性, 鼠龄 3~4 个月, 实验大鼠均常规分笼饲养, 环境温度为 22~26 ℃, 湿度 44%~50%, 每天光照时间为 12 h, 保持实验环境通风。

二、主要药物、试剂及实验器材

主要药物包括延参健胃胶囊 (天圣制药集团股份有限公司出品, 国药准字: Z20120004)、康复新液 (四川好医生攀西药业有限责任公司出品, 国药准字: Z51021834) 等; 主要试剂包括水杨酸钠 (天津永晟精细化工有限公司出品)、NO 试剂盒 (南京建成生物工程技术有限公司出品) 等; 主要实验器材包括 ZYS 型氦氖激光治疗机 (吉林科英激光技术有限责任公司出品)、PHS-3CW 型酸度计 (上海理达仪器厂出品)、华佗牌无菌针灸针 (苏州医疗用品厂出品, 规格为 0.30 mm×25.0 mm)、Forma 700 型超低温冰箱 (美国赛默飞世尔科技公司出品)、MK3 型酶标仪 (美国赛默飞世尔科技公司出品)、H-7650 型透射电镜 (日本日立公司出品) 等。

三、分组及处理

60 只 Wistar 大鼠经实验室适应性饲养 1 周后随机取 9 只大鼠纳入正常对照组, 常规自然喂养, 不予任何特殊干预, 其它大鼠参照文献^[7]介绍的方法将其制成 CAG 动物模型。制模过程中共有 6 只大鼠因灌胃不当而死亡, 制模结束前随机取 5 只大鼠进行胃黏膜大体观察及病理组织学检查, 如肉眼见胃黏膜出现不同程度充血、水肿、皱襞平坦、色泽灰白, 显微镜下见胃黏膜层变薄、固有腺体萎缩、炎性细胞浸润、胃体壁细胞减少、胞浆皱缩、上皮化生等典型 CAG 表现则确认造模成功^[7], 此时大鼠均停止造模给药。采用随机数字表法将上述制模成功大鼠分为模型组、药物组、针刺组及激光穴位组, 每组各 10 只。正常对照组、模型组大鼠置于大鼠固定器内制动, 每日 1 次, 每次制动 5 min。药物组大鼠给予延参健胃胶囊 (每千克体重 0.7 g) 和康复新液 (每千克体重 0.6 ml) 灌服; 针刺组、激光穴位组大鼠则置于大鼠固定器内, 参照《实验针灸学》^[8]相关定位标准, 针刺组采用毫针分别针刺左、右侧足三里穴, 针刺深度为 7 mm, 每侧针刺 1 min, 行平补平泻手法; 激光穴位组采用耦合光纤输出氦氖激光, 激光波长 632.8 nm, 输出功率 30 mW, 光斑直径 2 cm, 其光斑中心对准左、右侧足三里穴位点, 光纤头距皮肤 10 cm, 直接照射左、右侧足三里穴各 3 min。以上干预均为每日 1 次, 连续治疗 14 d。末次干预后禁食、不禁水持续 24 h 后进行体质量测量, 然后采用 10% 水合氯醛 (每千克体重 3.5 ml) 行腹腔注射麻醉, 断头取血 5 ml 并离心 (3000 r/min) 10 min, 分离血清置于 -80 ℃ 低温冰箱内贮存。实验大鼠随后剖腹、结扎贲门、摘出全胃, 先用 0.9% 生理盐水清洗, 再用滤纸吸干后沿胃大弯剪开胃腔, 然后用 5 ml 蒸馏水冲洗, 收集此稀释胃液, 再沿胃小弯取胃窦及部分胃体黏膜组织, 用 3% 戊二醛前固定, 4 ℃ 冰箱保存。采用硝酸还原酶法检测血清中 NO 含量, 具体操作步骤按试剂说明书进行。应用 PHS-3CW 型酸度计检测稀释胃液 pH 值, 具体测定步骤按仪器说明书进行。

四、电镜标本制作

将置于 4 ℃ 冰箱中的胃组织标本 (经戊二醛前固定) 取出, 经缓冲液洗涤后用 1% 四氧化锇后固定 1 h, 缓冲液洗涤漂洗, 逐级梯度丙酮脱水, 再置于

812 环氧树脂 (Epon-812) 中渗透、包埋,依次在包埋聚合仪中 37 ℃、45 ℃、60 ℃ 加热聚合各 24 h, 然后进行超薄切片 (片厚 70 nm), 经柠檬酸铅、醋酸铀双电子染色后置于 H-7650 型透射电镜下观察、照相。

五、统计学分析

本研究所得计量数据以 ($\bar{x} \pm s$) 表示, 采用 SPSS 17.0 版统计学软件包进行数据分析, 计量资料组间比较应用单因素方差分析, 组间多重比较应用最小显著差异法, $P < 0.05$ 表示差异具有统计学意义。

结 果

通过观察各组大鼠胃液 pH 值 (详见表 1) 及电镜下超微病理改变, 发现正常对照组大鼠胃液 pH 值较低, 其壁细胞胞膜结构完整、清晰, 细胞器丰富, 在胞浆内均匀分布 (图 1)。模型组大鼠胃液 pH 值较正常对照组明显升高 ($P < 0.05$), 其壁细胞核染色质凝聚, 部分胞膜结构缺失、溶解, 胞质中细胞器崩解、肿胀 (图 2)。药物组、针刺组及激光穴位组胃液 pH 值均较模型组有不同程度降低, 与正常对照组间差异均无统计学意义 ($P > 0.05$); 进一步分析发现, 药物组、激光穴位组胃液 pH 值与模型组间差异具有统计

学意义 ($P < 0.05$)。药物组、针刺组均见壁细胞胞膜结构基本完整, 胞浆内细胞器肿胀 (图 3、图 4); 激光穴位组可见完整的壁细胞胞膜结构及基本恢复正常的胞浆细胞器 (图 5)。

通过检测各组大鼠血清 NO 含量 (表 1), 发现正常对照组大鼠血清 NO 水平较低, 模型组大鼠血清 NO 水平较正常对照组明显升高 ($P < 0.05$)。药物组、针刺组及激光穴位组血清中 NO 含量均较模型组有不同程度降低, 其中针刺组、激光穴位组血清中 NO 水平与模型组间差异具有统计学意义 ($P < 0.05$)。药物组、针刺组及激光穴位组血清中 NO 水平与正常对照组间差异均无统计学意义 ($P > 0.05$)。

表 1 各组大鼠胃液 pH 值及血清中 NO 含量比较 ($\bar{x} \pm s$)

组别	只数	胃液 pH 值	血清中 NO 含量 ($\mu\text{mol/L}$)
正常对照组	9	2.162 $\pm$ 0.176 ^a	71.41 $\pm$ 39.45 ^a
模型组	10	3.073 $\pm$ 1.437	123.83 $\pm$ 31.56
药物组	10	2.165 $\pm$ 0.219 ^a	103.38 $\pm$ 34.37
针刺组	10	2.630 $\pm$ 0.583	91.59 $\pm$ 23.63 ^a
激光穴位组	10	2.163 $\pm$ 0.378 ^a	81.23 $\pm$ 26.19 ^a

注: 与模型组比较, ^a $P < 0.05$

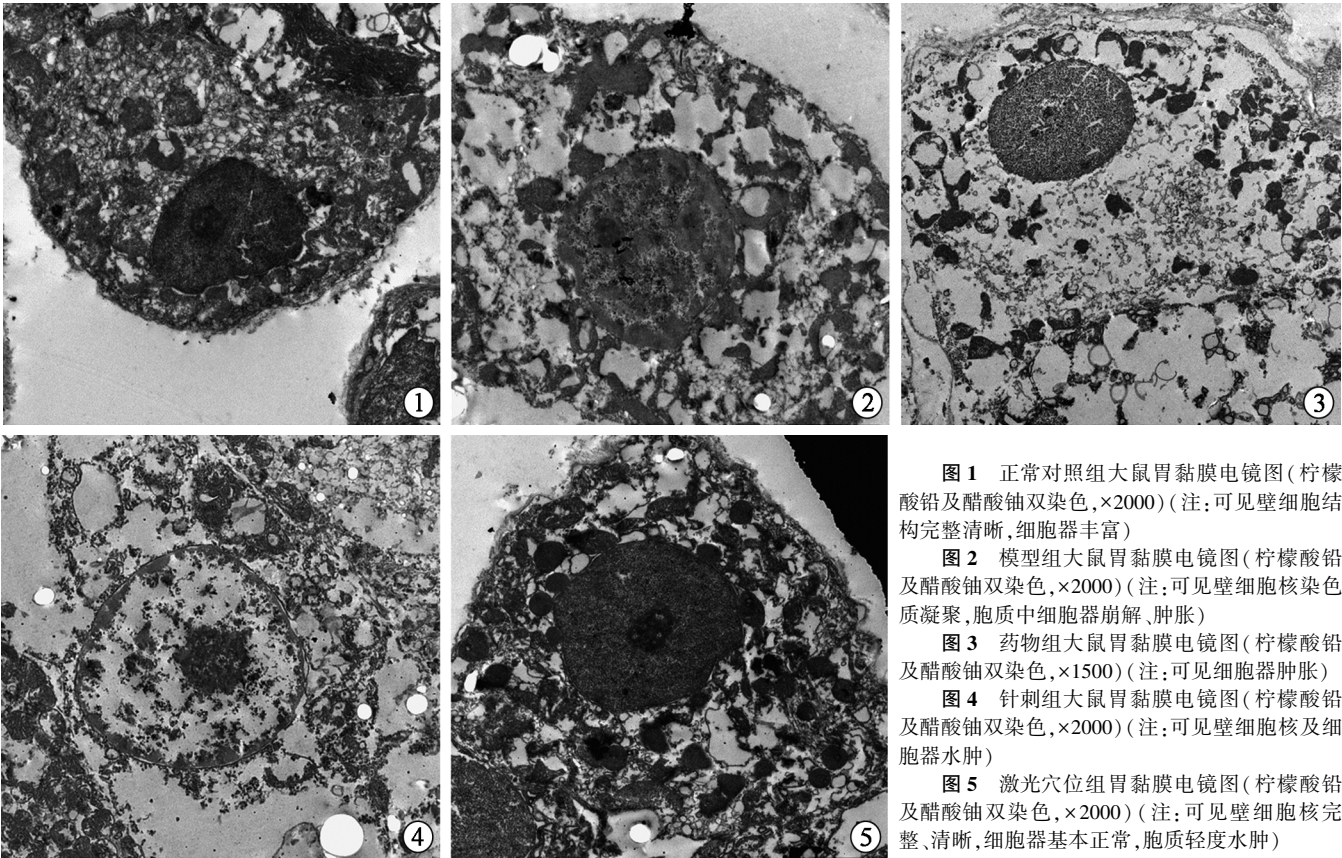


图 1 正常对照组大鼠胃黏膜电镜图 (柠檬酸铅及醋酸铀双染色, $\times 2000$) (注: 可见壁细胞结构完整清晰, 细胞器丰富)
图 2 模型组大鼠胃黏膜电镜图 (柠檬酸铅及醋酸铀双染色, $\times 2000$) (注: 可见壁细胞核染色质凝聚, 胞质中细胞器崩解、肿胀)
图 3 药物组大鼠胃黏膜电镜图 (柠檬酸铅及醋酸铀双染色, $\times 1500$) (注: 可见细胞器肿胀)
图 4 针刺组大鼠胃黏膜电镜图 (柠檬酸铅及醋酸铀双染色, $\times 2000$) (注: 可见壁细胞核及细胞器水肿)
图 5 激光穴位组大鼠胃黏膜电镜图 (柠檬酸铅及醋酸铀双染色, $\times 2000$) (注: 可见壁细胞核完整、清晰, 细胞器基本正常, 胞质轻度水肿)

讨 论

CAG 是临床常见慢性病,其病理改变基本特征是胃黏膜出血、充血、腺体萎缩、肠上皮化生、炎性细胞浸润等^[9]。根据患者临床表现,中医将 CAG 归属于“胃脘痛”、“痛满”等病症范畴,目前临床针对 CAG 患者主要给予药物及针灸治疗^[10],其中针灸治疗包括针刺、火针、温针、灸法、拔罐、穴位注射、耳压等,以针刺最为常用。现代研究表明,针刺对胃肠道系统的干预作用主要是改善胃电节律、调节胃泌素及其分泌、抑制胃酸分泌和保护胃黏膜等^[4,11]。氦氖激光单色性好、亮度大,照射机体组织会产生一系列生物学效应,主要有光化学效应、光刺激效应、光压强效应、光热效应、电磁效应等^[12-13],可对机体能量代谢及神经、内分泌、免疫网络调节系统产生良性影响^[12,14]。本研究将氦氖激光与穴位针刺相结合,应用氦氖激光直接照射特定穴位,据报道氦氖激光可穿透组织深度达 1.5 cm^[15],它不仅能穿透表皮组织发挥针刺样疗效,而且约有 40% 能量被表皮吸收并转化成热能及化学能,起到温灸作用,故氦氖激光照射穴位具备针和灸双重作用^[15-16]。

胃是一个复杂的机体器官,通过研究胃的分泌情况有助于判断胃部疾病。胃酸主要成分是盐酸,由壁细胞分泌,其主要作用是激活胃蛋白酶原,使其生成具有生物活性的胃蛋白酶;保持胃液合适 pH 值有利于胃蛋白酶发挥消化作用。相关研究指出,CAG 患者因胃腺体发生不同程度萎缩,胃液量减少,随着胃黏膜萎缩程度加重,其胃酸分泌呈持续降低趋势^[17-18],因此调节胃液 pH 值是治疗 CAG 的重要环节之一。本研究发现 CAG 大鼠胃黏膜壁细胞胞膜结构缺失,部分溶解、坏死,因其壁细胞数量下降,产酸减少,其胃液 pH 值增高,酸度降低。药物(如延参健胃胶囊、康复新液灌注)、针刺及激光穴位照射均有助于壁细胞恢复,促进胃酸分泌,其中药物治疗、激光照射足三里穴位能显著提高胃酸度;超微病理检查发现药物组、针刺组壁细胞结构基本完整,胞浆细胞器肿胀,而激光穴位组壁细胞结构完整,胞浆细胞器基本恢复正常。提示与药物及针刺疗法比较,激光照射足三里穴位对 CAG 具有更好疗效。

NO 是一种神经递质和信使分子,它与各类慢性疾病发生具有密切联系。相关研究表明,在炎症初期阶段 NO 主要发挥抗炎及抗组织损伤作用,但随着炎症进展,大量 NO 被释放并进入组织内,炎症病灶区被激活的炎性细胞在多种内、外因子刺激下,产生诱导型一氧化氮合酶(inducible nitric oxide synthase, iNOS),活性增强的 iNOS 又产生大量 NO 及超氧化物,生成过氧化亚硝酸盐,进而加重组织损伤,并且过度释放的 NO

还可诱发肿瘤形成^[19],可见超生理水平的 NO 对胃黏膜具有不利影响。本研究模型组大鼠血清 NO 含量较正常对照组明显升高($P<0.05$),提示 NO 浓度随胃黏膜萎缩程度加重而显著增加;而针刺及激光照射足三里穴均能显著降低 CAG 大鼠血清中 NO 含量($P<0.05$),并且以激光照射足三里穴对 NO 的下调作用尤为显著($P<0.05$),提示激光穴位照射可通过降低血清中 NO 含量来清除氧自由基,减轻或阻断脂质过氧化反应,提高胃黏膜抗氧化能力,从而增强胃黏膜防御功能,使 CAG 病情缓解甚至逆转。

综上所述,本研究结果表明,激光照射足三里穴可有效恢复壁细胞结构及功能,提高 CAG 大鼠胃酸分泌,降低血清中 NO 含量,对 CAG 病情具有较好的疗效及逆转作用;另外该疗法还具有无创、无痛、无感染风险等优点,值得临床进一步研究、推广。

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· 外刊撷英 ·

Physical activity and symptoms after concussion

BACKGROUND AND OBJECTIVE While some have argued that, acutely after a concussion, unrestricted physical activity may be detrimental to recovery, few studies have prospectively assessed the effect of behavior on patient outcome. This study was designed to better understand the association between physical activity and symptom recovery after an acute episode of concussion.

METHODS This prospective study included all patients diagnosed with a concussion seen in a sports medicine clinic between October of 2009 and July of 2011. Patients were assessed by physical examinations, with symptoms determined using the Post-Concussion Symptom Scale (PCSS), a 22-symptom inventory. During the initial clinic visit, patients were asked whether they had continued their regular exercise from the time of injury. At follow-up, the participants described their average levels of physical and cognitive activity since the previous clinic visit.

RESULTS A total of 364 patients were included in this study. The mean time to presentation after the injury was 11.8 days. Of those reporting a resolution of symptoms, the mean symptom duration was 48.9 days. While the initial PCSS scores and female gender were independently associated with symptom duration, physical activity after injury was not. For those ages 13 to 18 years, higher levels of physical activity after injury were associated with a shorter duration of symptoms.

CONCLUSION This prospective study found that physical activity after a concussion is not associated with symptom duration.

【摘自:Howell DR, Mannix RC, Quinn B, et al. Physical activity level and symptom duration are not associated after concussion. *Am J Sports Med*, 2016, 44(4): 1040-1046.】

Oral contraceptives and anterior cruciate ligament injury

BACKGROUND AND OBJECTIVE In a number of sports, women have higher injury rates of the anterior cruciate ligament (ACL) than do men. Some have postulated that estrogen may predispose women to ACL injuries. As progestins, the active compounds in oral contraceptives (OCs), disrupt the normal menstrual cycle, suppressing estrogen levels, this study assessed whether the use of oral contraceptives may be protective against ACL injury.

METHODS This case controlled study mined information from an insurance database collected from 2002 through 2012 concerning women ages 15 to 39 years. Subjects who underwent ACL reconstruction were compared to three matched controls. Cases had at least 90 days of OC use, with ACL injuries compared between those who did and those who did not use oral contraceptives.

RESULTS Of the 26.7 million women enrolled in the database, 12,819 underwent ACL reconstruction. The age group 15 to 18 years had the highest incidence of ACL reconstruction. Of those who underwent ACL reconstruction, 23.39% were users of OCs, compared with 22.82% among controls ($P<0.0001$). Importantly however, those with ACL injury had significantly higher percentages of enrollees labeled as high risk, receiving steroid injections, prescribed inhaled or oral steroids or antibiotics and diagnosed with asthma. Overall the adjusted odds ratio did not differ significantly between groups. However, in the 15 to 19 year age range, subjects undergoing ACL repair were 18% less likely to use oral contraceptives than were matched controls ($P<0.0001$).

CONCLUSION This population based study found that women 15 to 19 years of age who used oral contraceptives underwent 18% fewer ACL reconstructions than did matched non-users.

【摘自:Gray AM, Gugala Z, Baillargeon JG, et al. Effects of oral contraceptive use on anterior cruciate ligament injury epidemiology. *Med Sci Sports Exer*, 2016, 48(4): 648-654.】