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硕士学位论文

艾灸胃经穴治疗慢性萎缩性胃炎大鼠的代
谢组学研究

Metabonomics of Moxibustion and Gastric
Meridian in Treating Chronic Atrophic
Gastritis Rats

王亚东

指导教师姓名: 杨宗保副教授

专业名称: 针灸推拿学

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摘要

目的：研究艾灸干预胃经穴对慢性萎缩性胃炎（CAG）大鼠血清、胃、延髓和大脑皮质的影响，探讨艾灸胃经穴促进慢性萎缩性胃炎大鼠胃黏膜损伤修复的病理学变化和相关分子机制及代谢轮廓变化等。

方法：24只清洁级SD大鼠按随机数字表法随机分为4组，即正常组、模型组、艾灸胃经穴组、艾灸非穴位组，每组各6只。除正常组外，其余三组利用每日交替自由饮用氨水溶液（0.1%）和脱氧胆酸钠溶液（20mmol/L）结合饥饿障碍法制备大鼠慢性萎缩性胃炎模型，然后用艾灸分别刺激艾灸胃经穴组大鼠和艾灸非穴位组大鼠相关部位。收集大鼠的血清、胃、延髓和大脑皮质，采用智能生物显微镜下观察各组大鼠胃黏膜病理学变化、酶联免疫吸附测定法（enzyme linked immunosorbent assay ELISA）检测脑肠肽（P物质和Ghrelin蛋白）、核磁共振波氢谱（¹H NMR）技术等方法检测艾灸胃经穴对慢性萎缩性胃炎大鼠血清及胃黏膜、延髓和大脑皮质代谢物，获取一维¹H NMR谱，并利用多变量统计方法识别各组大鼠血清、胃黏膜、大脑皮质和延髓代谢轮廓的变化以及相关分子机制，探讨艾灸胃经穴促进慢性萎缩性胃炎大鼠胃黏膜损伤修复的细胞形态学变化和相关分子机制及代谢轮廓变化等。

结果：大鼠胃黏膜组织病理切片结果显示：正常组大鼠胃黏膜单层柱状上皮细胞和腺体排列整齐，胃黏膜完整性未遭到破坏，腺体和黏膜没有充血和水肿。模型组中可清楚地看到胃黏膜层的腺体细胞和腺管减少，在细胞和淋巴细胞中可见明显细胞浸润，细胞排列明显错乱。艾灸胃经穴组大鼠胃黏膜不同程度改善，腺体保持相对完整，炎症细胞浸润主要局限于胃黏膜表层。艾灸非穴位组中可观察到腺体形态不完整和细胞排列不规则。

摘要

各组大鼠胃黏膜组织的ELISA检测结果显示，与正常组相比，模型组大鼠胃黏膜组织中P物质和Ghrelin蛋白的表达水平显著降低 ($P < 0.05$)；与模型组相比，艾灸胃经穴组、艾灸非穴位组大鼠胃黏膜组织中P物质和Ghrelin蛋白的表达水平均发生显著上调 ($P < 0.05$)；与艾灸胃经穴组相比，艾灸非穴位组大鼠胃黏膜组织中P物质和Ghrelin蛋白的表达水平基本一致，无统计学差异 ($P > 0.05$)。

代谢组学检测结果显示，与正常组相比，模型组大鼠血清、胃、延髓和大脑皮质中代谢物的表达水平发生明显变化；与模型组相比，艾灸胃经穴组大鼠血清、胃、延髓和大脑皮质中代谢物的表达水平有明显的恢复，更靠近正常组大鼠的代谢水平，具有统计学意义 ($P < 0.05$)；与艾灸非穴位组进行比较，艾灸胃经穴组大鼠胃黏膜组织中代谢物的表达水平明显升高，更靠近正常组大鼠的代谢水平，具有统计学意义 ($P < 0.05$)。

结论：艾灸胃经穴可调节慢性萎缩性胃炎大鼠的细胞形态和相关的分子机制表达及代谢轮廓改变，说明艾灸胃经穴对慢性萎缩性胃炎具有确切的治疗作用。这对于以后揭示慢性萎缩性胃炎的确切发病机制及找出一种方便、安全有效、无毒副作用、廉价的治疗方法可能具有重要的参考价值。

关键词：慢性萎缩性胃炎；艾灸；胃经；代谢组学

Abstract

Objective: Through (^1H NMR), enzyme linked immunosorbent assay (ELISA), Western blotting (Western Blot WB) and microscopic observation of histopathology were performed by means of nuclear magnetic resonance spectroscopy (^1H NMR). Effect of moxibustion and stomach meridian on serum, urine and gastric tissue in rats with chronic atrophic gastritis, and to explore the metabolic and related molecular mechanism and cell morphological changes of gastric mucosal injury in rats with chronic atrophic gastritis.

Methods: 48 SD rats were randomly divided into 4 groups: normal group, model control group, moxibustion model acupoint group, moxibustion model group, 12 rats in each group. Each group was weighed and the rats were labeled with picric acid, and each rat was numbered accordingly. (0.1%) and deoxycholic acid solution (20mmol / L) were used to prepare the rat model of chronic atrophic gastritis [1], and then moxibustion was used in the other three groups except the normal group. Respectively, to stimulate the stomach group and non-acupoint group related parts. (^1H NMR), enzyme linked immunosorbent assay (ELISA), Western blotting (Western Blot WB) were used to detect the serum, urine and gastric tissue of rats. The effects of moxibustion and stomach meridian on the serum, urine and gastric tissue of rats with chronic atrophic gastritis were observed under the observation of pathology and histology. The one-dimensional ^1H NMR spectra were obtained and the multivariate statistical methods were used to identify each group. And the morphological changes of gastric mucosal cells were investigated. The metabolic and related molecular mechanisms and morphological changes of gastric mucosal injury were induced by moxibustion.

Results: The results of pathological biopsy showed that the normal tissue of the rats

Abstract

in the normal group was intact, the epithelium and glands were neat and uniform in size and shape. The cells were monolayer and had a cytoplasmic translocation / vacuole, glandular epithelium And the gland and the mucous membrane without dilation and blood stasis, mucosal myometrial hyperplasia, no pathological mitotic like; model group mucosa layer of the gland duct structure was disordered, duct morphology and size of the whole, Expansion of the duct, the size of the nucleus varies in the high magnification of the nucleus ratio increased, thick or loose reticular, nucleolus obvious; nucleus was rod-shaped or round, arranged uneven, visible mitotic; moxibustion The mucosal growth of the rats in the stomach meridian group was better than that in the model group, and the mucosal thickness and wrinkled wall were restored. The mucosa showed no obvious edema, atrophy, inflammation and neat gland, See the obvious atrophy, high magnification of the nucleus size is more uniform, neatly arranged, no pathological mitotic like; moxibustion non-acupoint group of mucosal cells significantly thinning atrophy, lamina propria and submucosal Can be seen more lymphocytes, plasma cells and mononuclear cell infiltration, mucosal muscle thickening, fibrous tissue hyperplasia, mucosal glandular atrophy, ductal morphology and size of the whole, the size of the nucleus, high magnification in the nuclear ratio Increased, thick stained, nucleolus obvious, arranged uneven, showing pathological mitotic like.

The injury index of gastric mucosa was the lowest in the normal group, and the score of gastric mucosal injury index in the model group was significantly higher than that in the control group ($P < 0.05$). The chronic atrophic ($P < 0.05$). Compared with

Abstract

the model group, the gastric mucosal injury index of moxibustion group was significantly lower than that of moxibustion group ($P < 0.05$), which indicated that moxibustion had significant effect on chronic atrophy ($P < 0.05$), which indicated that moxibustion and stomach acupoints were more effective in the treatment of moxibustion than the acupoint group. Repair of Gastric Mucosa in Chronic Atrophic Gastritis Injury.

The changes of blood flow in the gastric mucosa of rats in each group showed that the blood flow in the gastric mucosa of the model group was significantly decreased compared with the normal group. The gastric mucosal tissue of the moxibustion group Blood flow were significantly increased, of which moxibustion stomach meridian group, the most significant increase in gastric mucosal blood flow.

Compared with the model group, the levels of myo-Inositol, Glycine and isoleucine in the serum of the moxibustion were significantly higher than those of the normal group. Moxibustion creatinine, lactate, acetate, creatine, suine, glycine, choline, sugar beet The levels of metabolites such as betaine, succinate, and formate were also restored. The expression of Glycine, Inositol, Aspartic acid, Mannitol and Creatine Kinase in the stomach tissue of the moxibustion were increased, 2-Hydroxypropanoic acid, L-Glutamic Acid Hydrochloride, Ethyl 3-Hydroxybutyrate, DL-Methionine, Acetate, Glucose (Glucose), Isoleucine (Isoleucine) expression decreased, more tend to normal group of metabolic profile levels.

Conclusion: The changes of molecular and pathological changes of urine and gastric tissue in rats with chronic atrophic gastritis were detected by means of related

Abstract

molecular mechanisms and pathology, metabolic histology of nuclear magnetic resonance and other methods. Moxibustion and stomach meridian intervention in chronic atrophic gastritis model of metabolic changes in the law, further reveals the chronic atrophic gastritis injury and repair of the metabolic mechanism and foot yangming and stomach-related scientific connotation, from the perspective of material metabolism from the perspective of Ai Moxibustion of the meridian can regulate the metabolic pattern of chronic atrophic gastritis and its related molecular mechanism. It shows that moxibustion and stomach meridian have definite therapeutic effect on chronic atrophic gastritis, and the curative effect is closely related to the accuracy of acupoints, and also indirectly prove the existence of acupoints.

Keywords: chronic atrophic gastritis; Moxibustion; Metabolism of the stomach; metabolomic

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绪论

慢性萎缩性胃炎（chronic atrophy gastritis, CAG）是临床消化系统常见病、多发病、难治病之一，与胃癌特别是肠型胃癌的发生关系密切^[1]，被视为胃癌前病变^[2]。在慢性胃炎中约占10%~30%，以胃黏膜上皮和腺体萎缩，数目减少，胃黏膜变薄，黏膜基层增厚，或伴幽门腺化生和肠腺化生，或有不典型增生为特征^[3]。超微结构表明^[4]，在有害的外部环境影响下，慢性萎缩性胃炎可发展成胃癌。有长期随访调查表明，CAG每年的癌变率约为1.36%^[5]，严重威胁人类健康。常表现为上腹部隐痛、胀满、嗝气，食欲不振，或消瘦、贫血等，是一种多致病因素性疾病及癌前病变，是胃炎发展成胃癌的必经阶段，因此有效地防治慢性萎缩性胃炎已经成为预防难治性胃病的重要途径。

现代医学针对CAG尚无特效治疗方法，主要以对症治疗为主，如使用胃黏膜保护剂和PPI^[6]，对伴有轻度异型增生的CAG不做任何处理，中度异型增生者进行定期复查^[7]，重度异型增生者或疑为癌者采取外科手术治疗，戒烟忌酒，避免使用损害胃黏膜的药物如阿司匹林等，规律饮食^[8]，避免过热、过咸和辛辣食物，抗幽门螺杆菌治疗、抑制胆汁反流和改善胃动力等^[9]，可取得一定的疗效，但治疗方法僵化、单一，所用部分药物具有一定的副作用^[10]且部分患者症状改善不明显，效果不尽如人意^[11]，如长期使用PPI可引发一系列不良反应，甚至有致癌的风险。

艾灸是用艾叶等制成的易燃材料产生的温热作用刺激体表穴位或特定部位，通过激发经气来调整人体紊乱的生理生化功能，从而达到防病治病目的的一种外治方法^[12]，具有温散寒邪、温通经络、活血逐痹、回阳固脱、消瘀散结^[3]和防病

养生保健的功效^[13]。

本实验室多年研究艾灸治疗慢性萎缩性胃炎的具体机制,取得了一定的进展^[14,15],如发现艾灸胃经穴对胃黏膜损伤具有一定的保护作用等。根据本课题组长期大量的实验研究和临床一系列的相关报道,艾灸胃经穴对慢性萎缩性胃炎具有一定的防治效果,并已发现部分相关作用机制,但艾灸对慢性萎缩性胃炎的确切干预效应及其作用机制尚未得到充分揭示。大量的古代文献和现代实验及临床研究证明艾灸对慢性萎缩性胃炎有着良好的预防和治疗作用。本实验基于“足阳明经与胃相关具有特定代谢物质基础”假说,采用核磁共振代谢组技术和分子生物学相结合的方法,研究艾灸胃经穴对慢性萎缩性胃炎大鼠胃黏膜和血液、脊髓、大脑皮质等组织的代谢物变化规律,阐明艾灸胃经穴促进胃黏膜损伤修复的代谢物质基础,揭示足阳明经与胃粘膜损伤修复相关的代谢响应机制和科学内涵。

第一章 艾灸治疗慢性萎缩性胃炎的现代研究进展

1.1 艾灸治疗慢性萎缩性胃炎的文献研究

中医并无“慢性萎缩性胃炎”这一病名，根据其临床上表现的上腹饱胀、隐痛不适、痞闷、暖气等症状，可分属中医学中“胃脘痛”、“痞满”、“暖气”、“嘈杂”等范畴。《灵枢 经脉》谓：“足阳明之脉，是动则病，食则呕，胃脘痛”。《原病集》：“嘈杂乃心中似饥，烦杂不安，懒于饮食，谓之心嘈”。朱丹溪《丹溪心法》曰：“脾气不和，中央痞塞，皆土邪之所为也。”均强调本病病位在脾胃。

艾灸胃经穴对胃黏膜损伤有良好的调节作用，足阳明经脏腑络属上与脾胃相联系，《内经》记载该经有调理脾胃、助理运化等作用。因而，足阳明经是临床上治疗胃腑病证的首选经脉。足三里是胃经的合穴，同时又是胃的下合穴。《灵枢 邪气脏腑病形》载：“合治内腑”，概括了下合穴的主治功能。此外，《灵枢 四时气》载：“胃气逆则呕苦...取三里以下胃气逆”。明代徐凤《针灸大全·马丹阳天星十二穴并治杂病歌》也指出，足三里“善治胃中寒”，《四总穴歌》“肚腹三里留”则是对其主治作用的经典概括，由此可见，足三里与胃自古以来就被认为是存在着密切的联系。梁门穴，出《针灸甲乙经》，属足阳明胃经。位于脐上4寸、旁开2寸处。主治胃脘痛，呕吐，泄泻，胃下垂等。《针灸甲乙经》：“横木为梁，又迎前山岭为山梁，均含有横直之意。”《难经·五十七难》曰：“心之积曰伏梁，起于脐下，大如臂，上至心下。”又考其他方书，凡心阳失律，谷气寒凝，横胀塞满，类似潜伏之横梁者，可以取此，益阳气以灼阴邪，消寒滞而开痞郁。故称之为“梁门”。即破横亘之梁，而开通敞之门，亦以疗效而得名。

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