

## 证 的 研 究

## 慢性浅表性胃炎(脾胃湿热证)与水通道蛋白 4 表达关系的研究

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**摘要** 目的:从水液代谢来研究脾胃湿热证与水通道蛋白 4 (AQP4)表达的关系。方法:慢性浅表性胃炎患者 32 例,其中脾胃湿热证 20 例,脾气虚证 12 例;另 10 例正常人为对照。胃镜下取胃体上部粘膜,观察粘膜炎症情况,用免疫组化法、图像分析系统半定量 AQP4 的蛋白表达量。结果:脾胃湿热证胃粘膜的炎症明显重于脾虚证和正常人组( $P<0.05$ ,  $P<0.01$ );脾胃湿热证 AQP4 蛋白表达量强于脾虚证组( $P<0.01$ )和正常人组( $P<0.05$ );脾虚证蛋白表达量低于正常人组,但两组差异无显著性( $P>0.05$ )。结论:AQP 的异常表达可能是脾胃湿热证的发生机制之一。

**主题词** 胃炎/代谢 脾胃湿热/代谢 水通道蛋白质类/代谢

近年来关于脾实质的研究,证型上集中在脾虚证的研究,功能上集中在“脾主运化”、尤其“脾主运化水谷”的研究,而关于脾胃实证和“脾主运化水液”的研究较少。现代医学认为,水通道蛋白(AQP)是生物膜上特异性转运水的整合蛋白质,参与调节水平衡<sup>[1]</sup>。本文以水液代谢为切入点,研究脾胃湿热证与 AQP4 之间的关系。

## 1 临床资料

慢性浅表性胃炎的诊断标准参照中华医学会消化病学分会 2000 年井冈山会议制定的标准<sup>[2]</sup>,中医辨证脾胃湿热证和脾气虚证的诊断标准参照 2002 年国家药品监督管理局颁布的《中药新药临床研究指导原则》<sup>[3]</sup>,正常人的诊断标准为无心、肝、脾、肺、肾等系统疾病、常规实验室检查正常、胃粘膜胃镜征象和病理组织学基本正常。32 例慢性浅表性胃炎患者来源于广州中医药大学第一附属医院门诊部和住院部,辨证脾胃湿热证者 20 例,脾气虚证 12 例。另选取广州中医药大学第二临床医学院的 10 例符合正常人标准的作为对照。本科学生志愿者所有受试对象均签订知情同意书。

## 2 方法

2.1 取材:用日本 Olympus GIF-240XQ 型电子胃镜对所有受试对象进行检查,胃体距 EG 线 2cm 的大、小弯处分别钳取胃粘膜组织 4 块,每块 0.5cm×0.5cm,组织放入 4%多聚甲醛中。

2.2 普通病理组织学检测:组织固定后常规包埋切片,苏木素染色,伊红浸染,脱水、透明封片。3 位病理专科医师在未知病理诊断及同一设定条件下分析胃粘膜的病理改变。胃粘膜的炎症程度(轻度、中度、重度)参考文献[1]判定。

2.3 AQP4 的免疫组织学检测:组织经包埋切片后, H<sub>2</sub>O<sub>2</sub> 封闭,微波法热修复抗原,血清封闭液封闭,分别滴加羊抗人 AQP4 的多克隆抗体(美国 Santa Cruz 公司产品)、生物化二抗(武汉博士德公司产品)和 SABC, DAB 显色,苏木素衬染,透明封片。显微镜下观察,北航图像分析系统 3000 进行病理图像分析,以阳染强度×阳染面积作为阳性表达指数。

2.4 统计学方法:等级资料用 Ridit 分析和 K 独立样本检验,计量资料用方差分析。

## 3 结果

3.1 各组胃粘膜病变比较:正常人胃镜下胃粘膜呈均匀桔红色,粘膜光滑无充血,血管纹理清晰,病理组织学示上皮排列整齐,无明显炎性细胞浸润。慢性浅表性胃炎患者胃镜下呈现胃粘膜不同程度的充血水肿,粘液附着,甚至有少许浅糜烂灶,显微镜下示炎性细胞和淋巴细胞浸润。和脾虚证相比,脾胃湿热证患者胃镜下胃粘膜充血水肿明显,粘液增多,显微镜下也发现炎性细胞浸润的面积和深度均超过脾气虚证组( $P<0.05$ ),见表 1。

3.2 各组 AQP4 蛋白表达比较(免疫组化法):显微

表 1 各组胃粘膜炎症分级比较

| 组 别    | 例数 | 胃粘膜炎症分级(Ncase) |    |    |    |
|--------|----|----------------|----|----|----|
|        |    | 无              | 轻度 | 中度 | 重度 |
| 脾胃湿热证组 | 20 | 0              | 3  | 12 | 5  |
| 脾虚证组   | 12 | 0              | 6  | 5  | 1  |
| 正常人组   | 10 | 9              | 1  | 0  | 0  |

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表2 各组 AQP4 蛋白表达比较( $\bar{x} \pm s$ )

| 组别     | 例数 | AQP4 阳性表达指数(%)       |
|--------|----|----------------------|
| 脾胃湿热证组 | 20 | 209.00 $\pm$ 59.04   |
| 脾虚证组   | 12 | 126.67 $\pm$ 61.25** |
| 正常人组   | 10 | 164.00 $\pm$ 32.13*  |

与脾胃湿热证组比较, \*  $P < 0.05$ , \*\*  $P < 0.01$

镜下观察, AQP4 主要表达在粘膜固有层中, 分布在胃腺主细胞膜和壁细胞膜的基侧面。脾胃湿热证组阳染呈深棕黄色, 阳染面积比例大。脾虚证组阳染色呈浅棕色, 阳染面积小, 或阳染阴性。正常人组阳染强度中等, 阳染面积也较广。

阳染结果图像分析: 脾胃湿热证 AQP4 蛋白表达量强于脾虚证组 ( $P < 0.01$ ) 和正常人组 ( $P < 0.05$ ); 脾虚证蛋白表达量虽低于正常人组, 但两组差异无显著性 ( $P > 0.05$ ), 见表 2。

#### 4 讨论

脾(胃)为五脏六腑之中心。脾主运化, 主运化水液, 在水液代谢中起着重要作用, 可用“化”和“运”来概括, 即通过“脾的化”, 能够把水谷物的液态成分转化成水液, 能够把水液化生为唾、汗、尿、消化液及其它生理性液体; 通过“脾的运”, 能够把津液和多余的水液转输至肺、肾和全身。脾主运化水液的功能健旺, 可防止体内水液不正常的停滞, 起到维持体内津液平衡的作用。如脾(胃)运化水液失常, 易湿浊内生, 湿聚化热, 这也是脾胃湿热证的基本病机。可见, 脾胃湿热证与脾主运化水液、水液代谢密切相关。

近年研究发现, 在多种器官组织的细胞膜上存在着一种整合蛋白质, 即水通道蛋白, 它能特异性地介导水转运, 是水通过生物膜的主要方式<sup>[1]</sup>。1988 年首次从哺乳动物红细胞膜上分离出 AQP1<sup>[4]</sup>, 目前已成功分离 10 个亚型<sup>[5]</sup>。AQP 功能与其选择性分布有关, 对维持细胞、组织、器官及全身的水平衡起着决定性作用; 如果不同细胞膜上的 AQP 表达超过或低于正常水平, 都会影响水的分泌或吸收, 出现局部的或全身的水平衡紊乱<sup>[1]</sup>。

目前在消化道已发现有 7 种亚型的 AQP<sup>[6]</sup>。正常情况下, 人体每天通过唾液腺、胃、肝胆、胰腺、小肠、结肠等器官的上皮可吸收或者分泌的液体量超过 9L, 而这些液体在细胞内外的进出可能都与 AQP 有关。在人的胃体主要是 AQP4 表达, 且只在胃底腺主细胞和壁细胞的膜上表达, 功能是参与胃液的分泌, 和(或)参与维持壁的细胞正常容积<sup>[7]</sup>。AQP4 的作用强度可能与以下因素有关: 蛋白表达量、细胞膜上的排列方式、单位膜面积的分布强度等<sup>[8]</sup>。

本实验中我们发现, 胃粘膜 AQP4 在主细胞膜和壁细胞膜的基侧面表达, 而在细胞膜的顶部及粘液上皮细胞未见表达, 这些与 Misaka 等<sup>[7]</sup>的观察结果一致。脾胃湿热证组 AQP4 的蛋白表达要高于正常人组和脾虚证组 ( $P < 0.01$ ,  $P < 0.05$ ), 而脾虚证组则低于正常人组 ( $P > 0.05$ ); 同时还发现脾胃湿热证组胃粘膜的炎症程度要重于其它两组。

脾胃湿热证与水液代谢失衡密切相关。研究表明<sup>[9]</sup>, 脾胃湿热证的炎性介质增加(如组胺、5-羟色胺), 细胞代谢呈代偿性亢进状态, 胃泌素升高, 这些会引起胃粘膜炎症、胃液分泌增加。本实验中, 脾胃湿热证胃粘膜的 AQP4 升高, 可能会引起组织间隙和微血管的水分进入胃腺细胞或其它细胞, 引起局部水平衡紊乱, 参与炎症反应。而脾虚证则有线粒体能量传递低下, 胃粘膜分泌功能障碍<sup>[10]</sup>, 所以脾虚证的 AQP4 表达要低于脾胃湿热证和正常人。

脾胃湿热证和脾虚证一实一虚, 与水液代谢失衡都相关, AQP 表达也不同, 这提示 AQP 可以用来研究脾主运化水液的机制。

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## ABSTRACTS FROM ORIGINAL ARTICLES

### **Clinical Observation on 88 Cases with Ascites due to Cirrhosis of Hepatitis Treated by Xiaogu Ruanjian Bolus**

*Qi Zhongxi, Geng Lanshu, Gong Guiying, et al.*

**Objective:** To investigate therapeutic effect of Xiaogu Ruanjian Bolus on ascites due to cirrhosis of hepatitis. **Methods:** 133 cases of hepatitis with ascites due to cirrhosis were randomly divided into the treatment group ( $n=88$ ) treated by western medicine plus Xiaogu Ruanjian Bolus, and the control group ( $n=45$ ) treated by western medicine. Clinical symptoms and signs, hepatic functions, image, the time of ascitic extinction, therapeutic effects for ascites of different degrees, long-term therapeutic effect, and safety were investigated. **Results:** The treatment group, in remission of symptoms and signs, improvement of hepatic functions and image, the shortening of ascitic extinction time, therapeutic effect on severe ascites, and controlling relapse of ascites, was superior to the control group ( $P<0.05$ ,  $P<0.01$ ), and no obvious adverse effect was found. **Conclusion:** Xiaogu Ruanjian Bolus has a definite therapeutic effect on ascites due to cirrhosis of hepatitis.

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**Key words:** Hepatitis, type B/TCM therapy: hepatocirrhosis/TCM therapy: @Xiaogu Ruanjian Bolus

(Original article on page 347)

### **Clinical observation on 35 Cases of Ankylosing Spondylitis at Active Stage Treated by Qiangji Qingjie Decoction**

*Hu Yinqi, Tang Xianping, Chang Zhisui*

**Objective:** To observe therapeutic effect of Qiangji Qingjie Decoction on ankylosing spondylitis (AS) at active stage. **Methods:** 69 cases of AS at active stage were randomly divided into the treatment group ( $n=35$ ) treated with Qiangji Qingjie Decoction, and the control group ( $n=34$ ) treated with Shirebi Powder. Therapeutic effects were investigated after 3 consecutive therapeutic courses. **Results:** The controlling and markedly effective rate and the total effective rate were 62.86%, 88.57% in the treatment group, and 32.35%, 67.65% in the control group, with significant difference between two groups ( $P<0.01$ ). **Results:** Qiangji Qingjie Decoction could improve symptoms and signs of AS and significantly decreased blood sedimentation and C-reaction protein, and could arrest development of AS condition and improve functions of spinal joints.

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**Key words:** Spondylitis, ankylosing/TCM therapy: @Qiangji Qingjie Decoction

(Original article on page 350)

### **Study on Relation between Chronic Superficial Gastritis (Spleen and Stomach Damp-Heat syndrome) and Aquaporin 4 Expression**

*Zhou Zheng, Lao Shaoxian, Huang Zhixin, et al.*

**Objective:** To study relation between spleen and stomach damp-heat syndrome and aquaporin 4 (AQP4) expression. **Methods:** 32 cases of superficial gastritis including 20 cases of spleen and stomach damp-heat syndrome and 12 cases of spleen Qi-deficiency syndrome, and 10 normal persons were investigated. **Results:** Inflammation of gastric mucosa in the spleen and stomach damp-heat syndrome group was significantly more severe than that in the spleen deficiency group and the normal person group ( $P<0.05$ ,  $P<0.01$ ); AQP4 expression in the spleen and stomach damp-heat syndrome group was stronger than that in the spleen deficiency group ( $P<0.01$ ) and the normal person group ( $P<0.05$ ). **Conclusion:** Abnormal expression of AQP4 is possibly one of the mechanisms of spleen and stomach damp-heat syndrome.

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**Key words:** Gastritis/metabolism: damp-heat of spleen and stomach/metabolism: aquaporins/metabolism

(Original article on page 371)

### **Study on Correlativity of Syndrome Types of Allergic Rhinitis with Their Clinical Characteristics**

*Qiu Baoshan, Wang Shizhen, Zhong Yanping, et al.*

**Objective:** To Study clinical characteristics of different types of allergic rhinitis. **Methods:** TCM syndrome differentiation in 256 cases of allergic rhinitis were carried out by questionnaire and state of illness, acidophil of nasal secretion and serum immunoglobulin were determined. **Results:** Clinically, lung-Qi and spleen-Qi deficiency types of allergic rhinitis were common; lung-Qi deficiency type was mostly seasonal onset, with obvious rhinocnesmus, sneezing and nasal obstruction. Patients of spleen-Qi deficiency type were younger with more severe nasal obstruction and edema of nasal mucosa; patients of kidney Yang-deficiency type had longer duration of disease mostly with perennial onset, more nasal discharge, high serum IgE; patients of deficiency syndrome had mostly pale or reddish nasal mucosa; patients of the type of latent heat in the lung channel had less nasal discharge with red nasal mucosa as main. **Conclusion:** Allergic Rhinitis of Different syndrome types have corresponding characteristics in state of illness and clinical manifestations.

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**Key words:** Rhinitis, allergic, perennial/diagnosis

(Original article on page 373)