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乌梅丸通过 Notch 信号通路治疗寒热错杂证溃疡性结肠炎临床研究*

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摘要:目的 探讨乌梅丸通过 Notch 信号通路治疗寒热错杂证溃疡性结肠炎(UC)的临床疗效。方法 选取医院消化内科 2020 年 10 月至 2021 年 9 月收治的寒热错杂证 UC 患者 80 例,按随机数字表法分为观察组和对照组,各 40 例。对照组患者给予美沙拉嗪肠溶片口服治疗,观察组患者给予乌梅丸口服治疗,均连续治疗 8 周。结果 观察组患者的总有效率为 92.50%,显著高于对照组的 75.00% ($P < 0.05$)。两组患者治疗后的改良梅奥(Mayo)量表评分和 Baron 量表评分均显著降低,且显著低于对照组($P < 0.05$);血清细胞因子转化生长因子- β_1 (TGF- β_1)、胰岛素样生长因子 1(IGF-1)的水平均显著升高,且观察组显著高于对照组($P < 0.05$);D-乳酸、二胺氧化酶(DAO)的含量均明显降低,且观察组显著低于对照组($P < 0.05$);肠上皮组织中 Notch-1, Hes-1 mRNA 的表达水平均显著降低,Math-1 mRNA 的表达水平均显著升高,且观察组均改善更显著($P < 0.05$)。观察组的不良反应发生率为 5.00%,显著低于对照组的 15.00%($P < 0.05$)。结论 乌梅丸治疗寒热错杂证 UC 的临床疗效优于常规西药治疗,可有效促进患者的肠黏膜愈合,其作用机制可能与调节 Notch 信号通路,促进肠黏膜屏障修复和减轻炎症反应相关。

关键词: 乌梅丸; 溃疡性结肠炎; 寒热错杂证; Notch 信号通路; 临床疗效

Clinical Study of Wumei Pills in the Treatment of Ulcerative Colitis with Cold-Heat Complicated Syndrome Through Notch Signaling Pathway

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Abstract: Objective To investigate the clinical efficacy of Wumei Pills in the treatment of ulcerative colitis (UC) with cold-heat complicated syndrome through the Notch signaling pathway. **Methods** A total of 80 UC patients with cold-heat complicated syndrome admitted to the Department of Gastroenterology of the hospital from October 2020 to September 2021 were selected and divided into the observation group and the control group by the random number table method, with 40 cases in each group. The patients in the control group were given the oral treatment of Mesalazine Enteric-Coated Tablets, while the patients in the observation group were given the oral treatment of Wumei Pills. Both groups were treated continuously for 8 weeks. **Results** The total effective rate in the observation group was 92.50%, which was significantly higher than 75.00% in the control group ($P < 0.05$). After treatment, the modified Mayo scale score and Baron scale score in the two groups were significantly lower than those before treatment, and those in the observation group were significantly lower than those in the control group ($P < 0.05$); the levels of serum cytokines transforming growth factor- β_1 (TGF- β_1) and insulin-like growth factor-1 (IGF-1) in the two groups were significantly higher than those before treatment, and those in the observation group were significantly higher than those in the control group ($P < 0.05$); the contents of D-lactic acid and diamine oxidase (DAO) in the two groups were significantly lower than those before treatment, and those in the observation group were significantly lower than those in the control group ($P < 0.05$); the expression levels of Notch-1 and Hes-1 mRNA in intestinal epithelium in the two groups were significantly lower than those before treatment, while the expression levels of Math-1 mRNA in the two groups were significantly higher than those before treatment, and the improvement in the observation group was more significant ($P < 0.05$). The incidence of adverse reactions in the observation group was 5.00%, which was significantly lower than 15.00% in the control group ($P < 0.05$). **Conclusion** Wumei Pills in the treatment of UC with cold-heat complicated syndrome have better clinical efficacy than conventional Western medicine, which can effectively promote intestinal mucosal healing in patients. Its mechanism of action may be related to regulating the Notch signaling pathway, promoting intestinal mucosal barrier repair, and reducing inflammatory reactions.

Key words: Wumei Pills; ulcerative colitis; cold-heat complicated syndrome; Notch signaling pathway; clinical efficacy

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溃疡性结肠炎(UC)为慢性炎性浸润性肠病,可造成直肠和乙状结肠黏膜弥漫性炎性损伤,且病情反复,严重影响患者的生活质量^[1]。中医药具有不良反应少、复发率低等特点,在UC的治疗中具有独特优势,多种中药汤剂内服及外治法在改善UC症状、抑制肠黏膜炎症等方面发挥着重要作用^[2]。研究认为,UC是由感染、遗传、免疫异常、环境等多种因素共同作用引起的结肠黏膜结构和功能受损,进而诱发肠黏膜免疫炎症反应^[3]。Notch信号通路在肠上皮细胞分化和上皮再生过程中发挥关键作用,活化的Notch通路能破坏肠上皮黏膜屏障的防御功能,是导致UC病情加重的重要机制^[4]。乌梅丸具有寒温并用、清补兼施的特点,治疗消化系统疾病的疗效良好^[5]。既往动物实验结果显示,乌梅丸可通过抑制Notch信号通路相关靶基因的表达来减轻UC模型大鼠的肠黏膜损伤程度^[6-7]。本研究中探讨了乌梅丸通过Notch信号通路治疗寒热错杂证UC的临床疗效,以Notch信号通路为切入点,从细胞分子水平进一步明确乌梅丸在UC治疗中的分子机理。现报道如下。

1 资料与方法

1.1 一般资料

纳入标准:符合《炎症性肠病诊断与治疗的共识意见(2012年·广州)》中UC的诊断标准^[8],反复、持续发作6周及以上的腹痛、里急后重及脓血便,伴不同程度的全身表现;结肠镜下表现为肠黏膜充血、水肿、糜烂、溃疡、接触性出血等;UC活动期,病灶可见糜烂、溃疡、肉芽组织形成,慢性炎性细胞积聚、浸润,出现隐窝炎或隐窝脓肿;符合《溃疡性结肠炎中西医结合诊疗共识意见(2017年)》中寒热错杂证UC的辨证标准^[9];年龄18~65岁。本研究方案经医院医学伦理委员会审批(伦理审查受理号为20201215024),所有患者签署知情同意书。

排除标准:合并肠息肉、肠穿孔、肠梗阻等并发症及肛门疾病;急性感染性肠炎、肠易激综合征;合并严重肝、肾、造血系统等疾病;凝血功能障碍;1个月内使用过激素及免疫抑制剂。

病例选择与分组:选取我院消化内科2020年10月至2021年9月收治的寒热错杂证UC患者80例,按随机数字表法分为对照组和观察组,各40例。两组患者一般资料比较,差异无统计学意义($P > 0.05$),具有可比性。详见表1。

1.2 方法

对照组给予美沙拉嗪肠溶片(葵花药业集团佳木斯鹿灵制药有限公司,国药准字H19980148,规格为每片0.25g)口服,每次1片,每日4次。观察组给予乌梅丸口服,组方:乌梅15g,细辛5g,花椒10g,制附子10g,

表1 两组患者一般资料比较($n = 40$)

Tab. 1 Comparison of the patients' general data between the two groups ($n = 40$)

组别	性别 (男/女,例)	年龄 ($\bar{X} \pm s$,岁)	病程 ($\bar{X} \pm s$,年)	病情严重程度(例)		
				轻度	中度	重度
观察组	22/18	35.40 ± 5.39	4.27 ± 2.79	12	24	4
对照组	20/20	33.70 ± 4.28	4.80 ± 3.15	14	22	4
χ^2/t 值	0.0263	1.5622	0.7966	0.2279		
P值	0.8712	0.1223	0.4281	0.6331		

桂枝10g,干姜15g,人参10g,当归10g,黄连6g,黄柏10g,炙甘草6g,每日1剂,经常规水煎煮2次,分早、晚2次空腹温服。两组患者均治疗8周。

1.3 观察指标与疗效判定标准

观察指标:1)采用改良梅奥(Mayo)量表^[10]评估UC活动度,包括排便次数、便血情况、内镜表现、医师评价4项,分别计0分(正常)、1分(轻度)、2分(中度)、3分(重度)。2)采用Baron量表^[11]评估肠黏膜病变情况,依据黏膜病灶的内镜表现进行等级评定。黏膜无异常病变,为0级;黏膜仅见轻度炎症表现,为I级;黏膜轻度糜烂、溃疡,接触性出血,为II级;黏膜溃疡、糜烂数量超过3个,为III级;黏膜溃疡、糜烂广泛分布,为IV级。根据不同等级再计为0~4分。3)采集患者治疗前后的外周血,离心,取血清,采用酶联免疫吸附(ELISA)法测定血清中细胞因子转化生长因子- β_1 (TGF- β_1)、胰岛素样生长因子1(IGF-1)的水平,以及肠黏膜屏障功能指标二胺氧化酶(DAO)、D-乳酸的含量。4)内镜下取患者治疗前后肠上皮组织,提取总RNA,按反转录-聚合酶链式反应(RT-PCR)试剂盒步骤检测肠道组织Notch信号通路相关mRNA Notch-1, Hes-1, Math-1的表达水平。5)记录治疗过程中不良反应发生情况,包括恶心呕吐、头晕头痛、胃胀气等。

疗效判定^[12]:治愈,临床症状完全消失,内镜复查见肠黏膜炎症、溃疡和糜烂愈合;显效,临床症状基本缓解,内镜复查仅见肠黏膜轻度炎性反应,溃疡和糜烂愈合60%及以上;有效,临床症状较治疗前有一定程度缓解,内镜复查可见肠黏膜溃疡、糜烂愈合30%~59%;无效,临床症状和内镜复查表现较治疗前均无明显改善或较前加重。总有效=治愈+显效+有效。

1.4 统计学处理

采用SPSS 20.0统计学软件分析。计量资料以 $\bar{X} \pm s$ 表示,组内比较行配对样本 t 检验,组间比较行独立样本 t 检验;计数资料以率(%)表示,行 χ^2 检验。 $P < 0.05$ 为差异有统计学意义。

2 结果

结果见表2至表7。

表2 两组患者临床疗效比较[例(%), n = 40]

Tab. 2 Comparison of clinical efficacy between the two groups [case(%), n = 40]

组别	治愈	显效	有效	无效	总有效
观察组	9(22.50)	15(37.50)	13(32.50)	3(7.50)	37(92.50)
对照组	6(15.00)	13(32.50)	11(27.50)	10(25.00)	30(75.00)
χ^2 值					11.6969
P值					0.0085

表3 两组患者治疗前后Mayo、Baron量表评分比较($\bar{X} \pm s$, 分, n = 40)

Tab. 3 Comparison of Mayo and Baron scores between the two groups before and after treatment($\bar{X} \pm s$, point, n = 40)

组别	Mayo量表评分		t值	P值	Baron量表评分		t值	P值
	治疗前	治疗后			治疗前	治疗后		
观察组	8.07 ± 1.20	2.87 ± 0.73	23.4143	0.0000	2.92 ± 0.64	0.81 ± 0.37	18.0517	0.0000
对照组	8.11 ± 1.05	4.09 ± 1.13	16.4825	0.0000	2.95 ± 0.72	1.78 ± 0.54	8.2219	0.0000
t值	0.1587	5.7355			0.1970	9.3719		
P值	0.8743	0.0000			0.8444	0.0000		

表6 两组患者治疗前后Notch信号通路mRNA表达水平比较($\bar{X} \pm s$, n = 40)

Tab. 6 Comparison of mRNA expression levels of Notch signaling pathway between the two groups before and after treatment($\bar{X} \pm s$, n = 40)

组别	Notch-1 mRNA		t值	P值	Hes-1 mRNA		t值	P值	Math-1 mRNA		t值	P值
	治疗前	治疗后			治疗前	治疗后			治疗前	治疗后		
观察组	0.87 ± 0.13	0.51 ± 0.08	14.9161	0.0000	3.15 ± 0.16	1.34 ± 0.06	66.9911	0.0000	0.42 ± 0.05	2.73 ± 0.09	141.9022	0.0000
对照组	0.92 ± 0.10	0.66 ± 0.08	12.8405	0.0000	3.23 ± 0.20	2.19 ± 0.12	28.2010	0.0000	0.44 ± 0.07	1.57 ± 0.11	54.8131	0.0000
t值	1.9281	8.3853			1.9755	40.0694			1.4704	51.6193		
P值	0.0575	0.0001			0.0518	0.0000			0.1455	0.0000		

表7 两组患者不良反应发生情况比较[例(%), n = 40]

Tab. 7 Comparison of the incidence of adverse reactions between the two groups [case(%), n = 40]

组别	恶心呕吐	头晕头痛	胃胀气	合计
观察组	0(0)	1(2.50)	1(2.50)	2(5.00)
对照组	2(5.00)	3(7.50)	1(2.50)	6(15.00)
χ^2 值				5.5556
P值				0.0184

3 讨论

UC以直肠和乙状结肠黏膜糜烂、溃疡和弥漫性炎症等病理表现及慢性腹泻、黏液血便等消化道症状为主要特征,可伴不同程度的消瘦、贫血、乏力等全身表现,临床发病率较高,且病情迁延难愈^[13]。近年来,中药煎剂内服、中药保留灌肠、针灸等多种中医疗法作为UC的辅助治疗已在临床广泛应用^[14-15]。

UC中医归属“痢疾”“泄泻”“肠癖”等范畴,病位在大肠,发病与肝、脾密切相关,病机属虚实夹杂,以脾胃虚弱为本,湿毒内蕴为标,治疗当以寒温并用、清补兼施为原则。乌梅丸为治疗久泻、久痢的常用方,临床用于UC疗效确切^[16]。方中,乌梅养阴生津、涩肠止泻,为

表4 两组患者治疗前后血清细胞因子水平比较($\bar{X} \pm s$, ng/L, n = 40)

Tab. 4 Comparison of serum cytokine levels between the two groups before and after treatment($\bar{X} \pm s$, ng/L, n = 40)

组别	TGF- β_1		t值	P值	IGF-1		t值	P值
	治疗前	治疗后			治疗前	治疗后		
观察组	58.07 ± 7.29	93.52 ± 12.32	15.6620	0.0000	104.41 ± 16.12	183.70 ± 19.62	19.7486	0.0000
对照组	60.13 ± 9.06	77.09 ± 10.64	7.6756	0.0000	98.25 ± 20.23	147.09 ± 12.56	12.9721	0.0000
t值	1.1204	6.3834			1.5061	9.9392		
P值	0.2660	0.0000			0.1361	0.0000		

表5 两组患者治疗前后D-乳酸、DAO含量比较($\bar{X} \pm s$, n = 40)

Tab. 5 Comparison of D-lactic acid and DAO content between the two groups before and after treatment($\bar{X} \pm s$, n = 40)

组别	D-乳酸(mmol/L)		t值	P值	DAO(U/L)		t值	P值
	治疗前	治疗后			治疗前	治疗后		
观察组	6.09 ± 1.17	4.01 ± 0.65	9.8287	0.0000	7.34 ± 1.40	5.04 ± 1.15	8.0289	0.0000
对照组	6.03 ± 1.26	5.10 ± 0.72	4.0531	0.0001	7.80 ± 1.35	6.29 ± 1.22	5.2485	0.0000
t值	0.2207	7.1070			1.4959	4.7154		
P值	0.8259	0.0000			0.1387	0.0000		

君药;细辛、花椒通阳扶正、祛寒止痛,共为臣药;附子、桂枝、干姜温阳散寒、缓肝调脾,人参、当归养肝活血、补益脾气,黄连、黄柏清肝泄热、燥湿止痢,共为佐药;甘草健脾益气,调和诸药,为使药。全方清上温下,土木两调,故可用于治疗上热下寒、寒热错杂之证。现代药理学研究表明,乌梅丸可通过抗炎、解痉、止泻、促进黏膜修复等多种途径发挥对UC的治疗作用^[17-18]。本研究表明,观察组的总有效率显著高于对照组,治疗后的Mayo量表和Baron量表评分均显著低于对照组,表明乌梅丸治疗UC的疗效较美沙拉嗪更显著。

TGF- β_1 为TGF- β 超基因家族成员中占比最高的一类细胞因子,与UC炎症反应密切相关^[19]。IGF-1是由肝细胞合成并分泌的一类具有代谢效应和炎症调节功能的细胞因子,在UC的生理病理进程中发挥重要作用^[20]。本研究表明,观察组患者治疗后的血清TGF- β_1 和IGF-1水平均显著升高,表明乌梅丸能有效减轻炎症反应,从而减轻黏膜损伤。血清D-乳酸和DAO分别为肠道菌群代谢产物和肠黏膜中的细胞内酶,UC发生后引起肠黏膜通透性增加,使D-乳酸和DAO进入血液循环,加重病情^[21-22]。本研究表明,

观察组患者治疗后的血清D-乳酸和DAO的含量均显著降低,表明乌梅丸可通过提高肠黏膜功能,稳定肠黏膜屏障而发挥治疗作用。

Notch信号通路的活化在肠道上皮再生中发挥关键作用,能调节肠上皮细胞分化,并参与肠黏膜的修复过程^[23]。Notch过度活化时其靶基因Hes-1的表达可诱导肠上皮细胞向吸收细胞转化,Hes-1的表达又可抑制Math-1促使上皮细胞向分泌细胞转化的功能,进而减少杯状细胞等分泌细胞的数量,损害肠上皮黏膜屏障的防御功能,诱发炎症反应^[24]。本研究结果显示,观察组患者治疗后的Notch-1和Hes-1 mRNA表达水平均显著降低,Math-1 mRNA的表达水平显著升高,表明乌梅丸对UC患者Notch信号通路的过度激活具有一定调节作用,能抑制Notch-1,Hes-1 mRNA的表达,促进Math-1 mRNA的表达和肠上皮的再生,修复肠黏膜屏障的防御功能,减轻黏膜损伤,发挥对UC的治疗作用。本研究中观察组患者不良反应发生率显著低于对照组,表明安全性良好。

综上所述,乌梅丸治疗寒热错杂证UC的临床疗效优于常规西药治疗,可有效促进患者的肠黏膜愈合,其作用机制可能与调节Notch信号通路,促进肠黏膜屏障修复和减轻炎症反应相关。

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