

· 脂肪性肝病 ·

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化痰祛湿活血方对代谢相关脂肪性肝炎大鼠解整合素金属蛋白酶17/髓系细胞触发受体2介导的巨噬细胞胞葬的影响

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摘要: 目的 观察化痰祛湿活血方对代谢相关脂肪性肝炎(MASH)大鼠的治疗效果及作用机制。方法 选取60只SPF级SD大鼠随机分为空白对照组、模型A组(单纯性脂肪肝模型)、模型B组(MASH模型)、西药组(多烯磷脂酰胆碱, 143.64 mg/kg)、中药高剂量组(化痰祛湿活血方, 20.16 g/kg)和中药中剂量组(化痰祛湿活血方, 10.08 g/kg), 每组10只。除空白对照组外, 其余各组均给予高脂饲料。模型A组于第8周取材, 其余各组于第12周开始每日给药1次, 连续8周, 模型B组灌胃等体积的生理盐水, 于第20周取材。检测大鼠体重、肝湿重、肝指数; 采用微板法检测血清丙氨酸氨基转移酶(ALT)、天冬氨酸氨基转移酶(AST)、总胆固醇(TC)、甘油三酯(TG)、高密度脂蛋白胆固醇(HDL-C)、低密度脂蛋白胆固醇(LDL-C)和游离脂肪酸(FFA); 酶联免疫吸附法(ELISA)检测血清肿瘤坏死因子- α (TNF- α)、白细胞介素-1 β (IL-1 β)、IL-6、可溶性髓系细胞触发受体2(sTREM2); 苏木精-伊红染色、油红O染色观察肝组织病理变化; 免疫荧光法检测肝组织CD68⁺TREM2⁺细胞并计算巨噬细胞胞葬率; 实时荧光定量聚合酶链反应检测肝组织中鞘氨醇-1-磷酸(S1P)、鞘氨醇1磷酸酯受体1(S1PR1)、解整合素金属蛋白酶17(ADAM17)和髓系细胞触发受体2(TREM2)的mRNA表达水平; 免疫组织化学法检测肝组织中S1P、S1PR1、ADAM17和TREM2蛋白表达。符合正态分布的计量资料, 方差齐者采用单因素方差分析进行组间比较, 进一步两两比较采用LSD-*t*检验; 方差不齐者采用Welch's检验进行组间比较, 进一步两两比较则采用Tamhane's检验。不符合正态分布的计量资料组间比较采用Kruskal-Wallis *H*检验, 进一步两两比较采用Dunn's检验。结果 与空白对照组比较, 模型A组和模型B组的大鼠体重、肝湿重显著升高; 模型B组的肝指数显著升高(P 值均 <0.05)。HE染色结果显示, 模型A组大鼠肝组织呈现弥漫性大泡性脂肪变性, 模型B组大鼠肝组织显示大量气球样变性肝细胞, 小叶内及汇管区可见混合性炎症细胞浸润、轻度窦周纤维化。与空白对照组比较, 模型A组、模型B组大鼠NAS、油红O阳性面积显著升高(P 值均 <0.05); 且模型B组大鼠NAS、油红O阳性面积较模型A组显著升高(P 值均 <0.05)。与空白对照组比较, 模型A组、模型B组的血清TC、TG、LDL-C、FFA、IL-1 β 、IL-6、sTREM2水平均显著升高, 血清HDL-C水平显著降低, 模型B组的血清ALT、AST和TNF- α 水平显著升高(P 值均 <0.05); 且模型B组的血清ALT、AST、TC、TG、FFA、TNF- α 、IL-1 β 、IL-6和sTREM2水平较模型A组显著升高, 血清HDL-C水平较模型A组显著降低(P 值均 <0.05)。免疫荧光法结果显示, 与空白对照组比较, 模型A组巨噬细胞胞葬率显著升高($P < 0.05$); 模型B组巨噬细胞胞葬率显著低于模型A组($P < 0.05$)。实时荧光定量聚合酶链反应结果显示, 与空白对照组比较, 模型A组、模型B组TREM2的mRNA水平显著升高(P 值均 <0.05), 模型B组S1P、S1PR1的mRNA水平显著升高(P 值均 <0.05); 且模型B组S1PR1、TREM2的mRNA水平较模型A组升高(P 值均 <0.05)。免疫组织化学法结果显示, 与空白对照组比较, 模型A组和模型B组S1P、S1PR1、ADAM17蛋白表达水平显著升高, 模型A组TREM2蛋白表达水平显著升高(P 值均 <0.05); 且模型B组S1P、S1PR1和ADAM17蛋白表达水平较模型A组升高, TREM2蛋白表达水平较模型A组显著降低(P 值均 <0.05)。与模型B组比较, 各用药组大鼠体重、肝湿重和肝指数显著降低(P 值均 <0.05); 各用药组肝组织脂肪变及炎症损伤改善明显, NAS及油红O阳性面积显著降低(P 值均 <0.05); 血清ALT、AST、TC、TG、FFA、IL-1 β 、IL-6水平显著降低(P 值均 <0.05), 血清HDL-C水平显著升高($P < 0.05$), 中药高剂量组血清TNF- α 水平显著降低($P < 0.05$); 各用药组巨噬细胞胞葬率显著升高(P 值均 <0.05); 中药高剂量组、中药中剂量组ADAM17蛋白表达水平显著降低, 中药高剂量组TREM2蛋白表达水平显著升高(P 值均 <0.05)。结论 化痰祛湿活血方改善MASH大鼠肝脂质代谢及炎症水平可能与调控肝脏巨噬细胞胞葬有关。

关键词: 化痰祛湿活血方; 代谢相关脂肪性肝炎; 巨噬细胞; 胞葬作用

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Effect of Huatan Qushi Huoxue prescription on macrophage efferocytosis mediated by a disintegrin and metalloproteinase 17 and triggering receptor expressed on myeloid cells 2 in rats with metabolic dysfunction-associated steatohepatitis

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Abstract: Objective To investigate the therapeutic effect and mechanism of Huatan Qushi Huoxue prescription on rats with metabolic dysfunction-associated steatohepatitis (MASH). **Methods** A total of 60 specific pathogen-free Sprague-Dawley rats were randomly divided into blank control group, model A group, model B group, Western medicine group (polyene phosphatidylcholine, 143.64 mg/kg), high-dose Chinese medicine group (Huatan Qushi Huoxue prescription, 20.16 g/kg), and middle-dose Chinese medicine group (Huatan Qushi Huoxue prescription, 10.08 g/kg). All rats except those in the blank control group were given high-fat diet. Samples were collected from the model A group at week 8, and since week 12, the other groups were given the corresponding drug once a day for 8 consecutive weeks, with samples collected at week 20. Body weight, liver wet weight, and liver index were measured for all rats; the microplate method was used to measure the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and free fatty acids (FFA); ELISA was used to measure the serum levels of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and soluble triggering receptor expressed on myeloid cells 2 (sTREM2); HE staining and oil red O staining were performed to observe liver histopathological changes; immunofluorescence assay was used to measure CD68⁺TREM2⁺ cells in liver tissue and calculate the phagocytosis rate of macrophages; quantitative real-time PCR was used to measure the mRNA expression levels of sphingosine 1-phosphate (S1P), sphingosine 1-phosphate receptor 1 (S1PR1), a disintegrin and metalloproteinase 17 (ADAM17), and triggering receptor expressed on myeloid cells 2 (TREM2) in liver tissue, and immunohistochemistry was used to measure the protein expression levels of S1P, S1PR1, ADAM17, and TREM2 in liver tissue. A one-way analysis of variance was used for comparison of normally distributed continuous data with homogeneity of variance between groups, and the least significant difference *t*-test was used for further comparison between two groups; the Welch's test was used for comparison of normally distributed continuous data with heterogeneity of variance between groups, and the Tamhane's test was used for further comparison between two groups. The Kruskal-Wallis *H* test was used for comparison of non-normally distributed continuous data between groups, and the Dunn's test was used for further comparison between two groups. **Results** Compared with the blank control group, the model A group and the model B group had significant increases in body weight and liver wet weight, and the model B group had a significant increase in liver index (all $P < 0.05$). HE staining showed diffuse macrovesicular steatosis of liver tissue in the model A group and a large number of hepatocytes with ballooning degeneration in liver tissue in the model group B, with the presence of mixed inflammatory cell infiltration and mild perisinusoidal fibrosis in the lobules and the portal area. Compared with the blank control group, the model A group and the model B group had significant increases in NAS score and oil red O-positive area (all $P < 0.05$), and the model B group had significant increases in these two indicators than the model A group (both $P < 0.05$). Compared with the blank control group, the model A group and the model B group had significant increases in the serum levels of TC, TG, LDL-C, FFA, IL-1 β , IL-6, and sTREM2 and a significant reduction in the serum level of HDL-C, and the model B group had significant increases

in the serum levels of ALT, AST, and TNF- α (all $P<0.05$); compared with the model A group, the model B group had significant increases in the serum levels of ALT, AST, TC, TG, FFA, TNF- α , IL-1 β , IL-6, and sTREM2 and a significant reduction in the serum level of HDL-C (all $P<0.05$). Immunofluorescence assay showed that compared with the blank control group, the model A group had a significant increase in the phagocytosis rate of macrophages ($P<0.05$), while the model B group had a significantly lower phagocytosis rate of macrophages than the model A group ($P<0.05$). Quantitative real-time PCR showed that compared with the blank control group, the model A group and the model B group had a significant increase in the mRNA expression level of TREM2, and the model B group had significant increases in the mRNA expression levels of S1P and S1PR1 (both $P<0.05$); moreover, compared with the model A group, the model B group had significant increases in the mRNA expression levels of S1PR1 and TREM2 (both $P<0.05$). Immunohistochemistry showed that compared with the blank control group, the model A group and the model B group had significant increases in the protein expression levels of S1P, S1PR1, and ADAM17, and the model A group had a significant increase in the protein expression level of TREM2 (all $P<0.05$); compared with the model A group, the model B group had significant increases in the protein expression levels of S1P, S1PR1, and ADAM17 and a significant reduction in the protein expression level of TREM2 (all $P<0.05$). Compared with the model B group, each medication group had significant reductions in body weight, liver wet weight, and liver index (all $P<0.05$); each medication group had significant improvements in hepatic steatosis and inflammatory damage, with significant reductions in NAS score and oil red O-positive area (all $P<0.05$); each medication group had significant reductions in the serum levels of ALT, AST, TC, TG, FFA, IL-1 β , and IL-6 (all $P<0.05$) and a significant increase in the serum level of HDL-C ($P<0.05$), and the high-dose Chinese medicine group had a significant reduction in the serum level of TNF- α ($P<0.05$); each medication group had a significant increase in the phagocytosis rate of macrophages (all $P<0.05$); the high- and middle-dose Chinese medicine groups had a significant reduction in the protein expression level of ADAM17, and the high-dose Chinese medicine group had a significant increase in the protein expression level of TREM2 (all $P<0.05$). **Conclusion** Huatan Qushi Huoxue prescription improves lipid metabolism and inflammation in the liver of MASH rats by regulating hepatic macrophage phagocytosis.

Key words: Huatan Qushi Huoxue Formula; Metabolic Dysfunction-Associated Steatohepatitis; Macrophage; Efferocytosis

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代谢相关脂肪性肝炎(metabolic dysfunction-associated steatohepatitis, MASH)是代谢相关脂肪性肝病(metabolic dysfunction-associated fatty liver disease, MAFLD)的中间阶段,其病理特征是肝细胞脂肪变性、炎症以及氧化应激损伤,严重者可进一步进展为肝硬化甚至肝癌^[1]。据统计,2020年我国MASH患病人数为3 870万,预计到2030年将增长至5 550万^[2]。目前,全球首个治疗MASH成人患者的药物瑞司美替罗已获美国食品药品监督管理局的批准上市,但其仅能使25%~30%的患者获益,其长期安全性仍有待评估,且价格昂贵,尚未在国内上市^[3]。因此,考虑到长期治疗的需要,需平衡药物的有效性、安全性、耐受性和成本。中医药在治疗MASH方面表现出独特优势,具有良好的安全性,且前期研究也证实中医药可以有效治疗MASH^[4-5]。痰湿阻络,久而成瘀,痹阻于肝络是MASH的中医病机^[6],赵文霞教

授依据MASH的基本病机创制了化痰祛湿活血方,用于治疗MASH。临床研究显示,化痰祛湿活血方可提高MASH患者肝/脾CT值比值,降低体重指数及血清游离脂肪酸(free fatty acids, FFA)、甘油三酯(triglyceride, TG)和丙氨酸氨基转移酶(alanine aminotransferase, ALT)水平^[7]。

巨噬细胞胞葬功能受损致凋亡肝细胞持续堆积是MASH发生发展的核心驱动因素^[8],当FFA大量聚集时,持续的肝细胞凋亡未能被及时清除,则会诱发肝细胞坏死,坏死的肝细胞释放损伤相关分子模式,激活肝脏巨噬细胞中的解整合素金属蛋白酶17(adisintegrin and metalloprotease 17, ADAM17),促使髓系细胞触发受体2(triggering receptor expressed on myeloid cells 2, TREM2)蛋白裂解,导致巨噬细胞胞葬作用受损,凋亡肝细胞在肝脏中异常堆积,形成恶性循环回路,产生慢性炎症,加

剧MASH的发生发展^[9]。本研究以肝巨噬细胞胞葬作用为切入点,探讨化痰祛湿活血方改善MASH的作用机制,旨在为临床MASH治疗提供科学依据。

1 材料与方法

1.1 实验动物与饲料 60只140~170 g SPF级SD大鼠购于浙江维通利华实验动物技术有限公司[实验动物质量合格证编号:20230222Aazz0619000986,生产许可证号:SCXK(浙)2019-0001,使用许可证号:SYXK(豫)-2021-0015]。所有实验动物于河南中医药大学动物实验中心饲养。普通饲料由河南中医药大学动物实验中心提供,高脂饲料(热量含量为脂肪42%、蛋白质14%、碳水化合物44%、胆固醇0.2%)购于江苏省协同医药生物工程有限公司(批号:20230204)。

1.2 药物组成与制备 化痰祛湿活血方颗粒剂由泽泻、海藻、决明子、郁金、丹参、山楂、柴胡和水飞蓟组成,委托江苏省江阴天江药业有限公司制备,经过中药饮片提取、浓缩、喷雾干燥、过筛混合及制粒后,制成12 g/袋规格的颗粒剂(批号:2205302)。多烯磷脂酰胆碱胶囊购自赛诺菲(北京)制药有限公司(批号:H20059010)。

1.3 试剂 ALT、天冬氨酸氨基转移酶(aspartate aminotransferase, AST)、总胆固醇(total cholesterol, TC)、TG、高密度脂蛋白胆固醇(high density lipoprotein cholesterol, HDL-C)、低密度脂蛋白胆固醇(low-density lipoprotein cholesterol, LDL-C)、FFA测定试剂盒均购于南京建成生物工程研究所(货号分别为C009-2-1、C010-2-1、A111-1-1、A110-1-1、A112-1-1、A113-1-1、A042-2-1);肿瘤坏死因子- α (tumour necrosis factor- α , TNF- α)、白细胞介素-1 β (interleukin-1 β , IL-1 β)、IL-6 ELISA试剂盒均购于Elabscience(货号分别为E-EL-R2856、E-EL-R0012、E-EL-R0015);可溶性髓系细胞触发受体2(soluble triggering receptor expressed on myeloid cells 2, sTREM2)ELISA试剂盒购于FineTest(货号:ER2057);HE高清恒染试剂盒、油红染液、RNA提取液、SweScript All-in-One RT SuperMix for qPCR(One-Step gDNA Remover)、2 \times Universal Blue SYBR Green qPCR Master Mix、CD68一抗均购于Servicebio(货号分别为G1076、G1015、G3013、G3337、G3326、GB113109-100);TREM2一抗购于Bioss(货号:bs-2723R);ADAM17一抗购于Gene Tex(货号:GTX101358);鞘氨醇-1-磷酸(sphingosine-1-phosphate, SIP)一抗购于Affinity Biosciences(货号:DF4159);鞘氨醇1磷酸酯受体1

(recombinant sphingosine 1 phosphate receptor 1, S1PR1)一抗购于proteintech(货号:55133-1-AP)。

1.4 仪器 病理切片机(德国Leica)、光学显微镜(日本Olympas)、荧光显微镜(日本Olympas)、酶标仪(瑞士TECAN)、紫外可见分光光度计(美国Thermofisher)、热循环仪(美国Thermofisher)、荧光定量聚合酶链反应(polymerase chain reaction, PCR)仪(美国Thermofisher)、凝胶成像分析系统(美国BIO-RAD)。

1.5 实验方法

1.5.1 造模、分组及给药 将60只SPF级SD大鼠随机分为6组,每组10只:空白对照组、模型A组、模型B组、西药组、中药高剂量组和中药中剂量组。空白对照组予普通饲料,其余5组予高脂饲料。模型A组为代谢相关单纯性脂肪肝(metabolic dysfunction-associated fatty liver, MAFL)大鼠模型,模型B组为MASH大鼠模型。模型A组大鼠于第8周取材,其余各组从第12周起开始灌胃相应药物。根据人和动物间体表面积折算的等效剂量计算灌胃剂量(60 kg成人和大鼠的换算系数是6.3),西药组灌胃多烯磷脂酰胆碱胶囊混悬液143.64 mg/kg,中药高剂量组、中药中剂量组分别灌胃化痰祛湿活血方颗粒剂溶液20.16 g/kg、10.08 g/kg,空白对照组、模型B组灌胃等体积的生理盐水,每日灌胃1次,灌胃至第20周取材。

1.5.2 采集标本 取材前大鼠禁食和禁水12 h。大鼠称重后,采用10%水合氯醛溶液(0.3 mL/100 g)腹腔注射的方式麻醉,暴露腹主动脉,使用采血针从腹主动脉处采血。取2块肝组织分别放于2管10%中性缓冲福尔马林溶液中固定,剩余肝组织分放至冻存管中,置于液氮罐中冷冻后转移至-80℃冰箱。离心血液,取上清液,冻存于-80℃冰箱备用。

1.5.3 称取大鼠肝湿重、计算肝指数 称取并记录每只大鼠体重及肝湿重,肝指数=(肝湿重/大鼠体重) \times 100%。

1.5.4 微板法检测大鼠血清肝脏酶学、血脂 按照对应试剂盒步骤检测ALT、AST、TC、TG、HDL-C、LDL-C和FFA水平。

1.5.5 ELISA法检测血清炎症因子、sTREM2水平 按照试剂盒说明书检测TNF- α 、IL-1 β 、IL-6和sTREM2水平。

1.5.6 肝组织病理学检测 取固定后肝组织,脱水与透明,石蜡包埋,切片;苏木精-伊红染色(hematoxylin and eosin Staining, HE染色):脱蜡、苏木精染核、分化、反蓝、

伊红染色胞浆、脱水与透明、封片,由2名病理医师背靠背采用光学显微镜进行非酒精性脂肪性肝病活动度评分(non-alcoholic fatty liver disease activity score, NAS)^[10],拍片;油红O染色:4%多聚甲醛固定、石蜡包埋、切片、脱蜡、水化、油红O染色、苏木精复染、封片、观察、摄片,每个样本随机采集5个区域,采用Image J软件测量每张区域的油红O阳性区域和区域面积,计算油红O阳性面积比率=油红O阳性面积/区域面积,并取5个随机区域的平均值为该样本的最终油红O阳性面积比率。从每组大鼠中随机选取5只大鼠的肝组织样本进行组间比较。

1.5.7 免疫荧光法检测巨噬细胞胞葬率 取石蜡包埋的肝组织并进行切片,脱蜡,抗原修复,封闭,一抗孵育(CD68、TREM2稀释比例分别为1:300、1:200),二抗孵育,DAPI染液孵育,封片,拍照;采用Image J软件测量CD68⁺TREM2⁺细胞数、CD68⁺细胞数,并采用公式计算巨噬细胞胞葬率(%)=CD68⁺TREM2⁺细胞数/CD68⁺细胞数×100%。从每组大鼠中随机选取5只大鼠的肝组织样本进行组间比较。

1.5.8 实时荧光定量PCR检测肝组织中巨噬细胞胞葬相关基因表达 按照试剂盒步骤提取肝组织RNA,测RNA浓度,逆转录(42℃孵育15 min,85℃孵育5 min),根据引物序列(表1)进行PCR扩增,从PCR仪器上导出实验结果,计算S1P、S1PR1、ADAM17和TREM2基因及内参基因的表达水平。从每组大鼠中随机选取6只大鼠的肝组织样本进行组间比较。

表1 引物序列
Table 1 Primer information

| 基因 | 引物序列(5'-3') | 碱基数 |
|--------|-----------------------------|-----|
| GAPDH | 上游 CTGGAGAAACCTGCCAAGTATG | 22 |
| | 下游 GGTGGAAGAATGGGAGTTGCT | 21 |
| S1P | 上游 TCATCACGTCCCCTGAAAAGAG | 22 |
| | 下游 CAAAAACAGCAACCCTGACATTAG | 24 |
| S1PR1 | 上游 GCTGAACATCGGAGTGGAGAAG | 22 |
| | 下游 GAGCCACAAACATACTTCCTTCC | 23 |
| TREM2 | 上游 CCAAGCCCTCAACACCACA | 19 |
| | 下游 ACCGTGCTCCCATTCTGCTT | 20 |
| ADAM17 | 上游 AAGGGATCTACAGTCTGCGACA | 22 |
| | 下游 CCTAGAGTCAGGCTCACCAACC | 22 |

注:GAPDH,甘油醛-3-磷酸脱氢酶;S1P,鞘氨醇-1-磷酸;S1PR1,鞘氨醇1磷酸酯受体1;TREM2,髓系细胞触发受体2;ADAM17,解整合素金属蛋白酶17。

1.5.9 免疫组化法检测肝组织中巨噬细胞胞葬相关蛋白表达 取石蜡包埋的肝组织并进行切片,使用3%过氧化氢的甲醇洗涤液处理,滴加一抗(S1P、S1PR1、ADAM17和TREM2稀释比例均为1:200)在4℃下孵育过夜,PBS缓冲液洗涤,二抗孵育,洗涤,DAB底物处理,封片,观察、拍片。每个样本随机采集5个区域,采用Image J软件测量每个区域的累积光密度值和区域面积,计算平均光密度值=累积光密度值/区域面积,并取5个随机区域的平均值为该样本的最终平均光密度值。从每组大鼠中随机选取5只大鼠的肝组织样本,进行组间统计学比较。

1.6 统计学方法 采用SPSS 22.0软件进行数据统计分析,符合正态分布的计量资料采用 $\bar{x}\pm s$ 表示,方差齐者采用单因素方差分析进行组间比较,进一步两两比较采用LSD-*t*检验;方差不齐者采用Welch's检验进行组间比较,进一步两两比较则采用Tamhane's检验;不符合正态分布的计量资料采用 $M(P_{25} \sim P_{75})$ 描述,组间比较采用Kruskal-Wallis *H*检验,进一步两两比较采用Dunn's检验并校正检验水准。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 化痰祛湿活血方对大鼠体重、肝湿重和肝指数的影响 与空白对照组比较,模型A组的体重、肝湿重显著升高(P 值均 < 0.05),模型B组的体重、肝湿重和肝指数显著升高(P 值均 < 0.05);与模型B组比较,模型A组、西药组、中药高剂量组和中药中剂量组的体重、肝湿重和肝指数均显著降低(P 值均 < 0.05);中药高剂量组的肝湿重显著低于西药组($P < 0.05$)(表2,图1)。

2.2 化痰祛湿活血方对大鼠肝组织病理的影响 模型A组肝组织肝小叶结构尚完整,呈现弥漫性大泡性脂肪变性,肝细胞体积明显增大,中央静脉及汇管区结构清晰,未见明显紊乱,小叶内及汇管区偶见散在单个核细胞,无灶状或片状炎症浸润;模型B组肝组织显示肝索结构紊乱,肝窦受压变窄,肝细胞体积显著增大,胞浆内充满大小不等的圆形空泡,视野内散在大量气球样变性肝细胞,部分细胞核被挤向边缘,小叶内及汇管区可见灶状或片状混合性炎症细胞浸润,以单核细胞、淋巴细胞为主,局部出现点状肝细胞坏死,汇管区及肝小叶内可见轻度窦周纤维化;给药组均较模型B组肝组织病理有改善,以中药高剂量组改善最为显著(图1)。HE染色显示,与空白对照组比较,模型A组、模型B组的NAS显著升高;与模型B组比较,模型A组、西药组、中药高剂量

组和中药中剂量组的NAS显著降低;中药高剂量组的NAS显著低于西药组(P 值均 <0.05)(表3,图1)。油红O阳性面积显示,与空白对照组比较,模型A组、模型B组的油红O阳性面积显著升高;与模型B组比较,模型A组、西药组、中药高剂量组和中药中剂量组的油红O阳性面积显著降低;中药高剂量组油红O阳性面积较西药组、中药中剂量组显著降低(P 值均 <0.05)(表3,图1)。上述研究结果表明,化痰祛湿活血方可改善MASH大鼠肝组织病理损伤。

2.3 化痰祛湿活血方对大鼠血清肝脏酶学、血脂的影响 与空白对照组比较,模型B组的血清ALT、AST水平显著升高;与模型B组比较,模型A组、西药组、中药高剂量组和中药中剂量组的血清ALT、AST水平显著降低;中药高剂量组的血清ALT、AST水平显著低于西药组(P 值均 <0.05)。

与空白对照组比较,模型A组及模型B组的血清

TC、TG、LDL-C和FFA水平显著升高,HDL-C水平显著降低;与模型B组比较,模型A组、西药组、中药高剂量组和中药中剂量组的血清TC、TG和FFA水平显著降低,HDL-C水平显著升高;中药高剂量组的血清FFA水平显著低于西药组、中药中剂量组,HDL-C水平显著高于西药组、中药中剂量组(P 值均 <0.05)(表4)。

2.4 化痰祛湿活血方对大鼠炎症因子及sTREM2的影响 与空白对照组比较,模型B组的血清TNF- α 水平显著升高,模型A组、模型B组的血清IL-1 β 、IL-6水平显著升高;与模型B组比较,模型A组、中药高剂量组的血清TNF- α 、IL-1 β 和IL-6水平显著降低,西药组、中药中剂量组的血清IL-1 β 、IL-6水平显著降低;中药高剂量组的血清TNF- α 、IL-1 β 水平较西药组显著降低,TNF- α 、IL-1 β 、IL-6水平较中药中剂量组显著降低(P 值均 <0.05)。

与空白对照组比较,模型A组、模型B组的血清sTREM2水平显著升高;与模型B组比较,模型A组、中

表2 各组大鼠体重、肝湿重、肝指数

Table 2 Rat weight, liver wet weight, and liver index of each group of rats

| 组别 | 动物数(只) | 体重(g) | 肝湿重(g) | 肝指数(%) |
|--------|--------|------------------------------------|----------------------------------|-------------------------------|
| 空白对照组 | 10 | 542.80 \pm 27.48 | 16.23 \pm 1.27 | 2.99 \pm 0.22 |
| 模型A组 | 10 | 593.50 \pm 10.46 ¹⁾²⁾ | 20.43 \pm 2.55 ¹⁾²⁾ | 3.45 \pm 0.46 ²⁾ |
| 模型B组 | 10 | 742.70 \pm 79.01 ¹⁾ | 39.80 \pm 10.46 ¹⁾ | 5.30 \pm 0.95 ¹⁾ |
| 西药组 | 10 | 622.50 \pm 62.62 ²⁾ | 22.23 \pm 2.52 ²⁾³⁾ | 3.57 \pm 0.22 ²⁾ |
| 中药高剂量组 | 10 | 583.60 \pm 21.02 ²⁾ | 18.68 \pm 1.90 ²⁾ | 3.20 \pm 0.28 ²⁾ |
| 中药中剂量组 | 10 | 602.80 \pm 51.71 ²⁾ | 22.09 \pm 3.46 ²⁾ | 3.67 \pm 0.51 ²⁾ |
| F 值 | | 12.954 | 20.209 | 15.394 |
| P 值 | | <0.01 | <0.01 | <0.01 |

注:与空白对照组比较,1) $P<0.05$;与模型B组比较,2) $P<0.05$;与中药高剂量组比较,3) $P<0.05$ 。

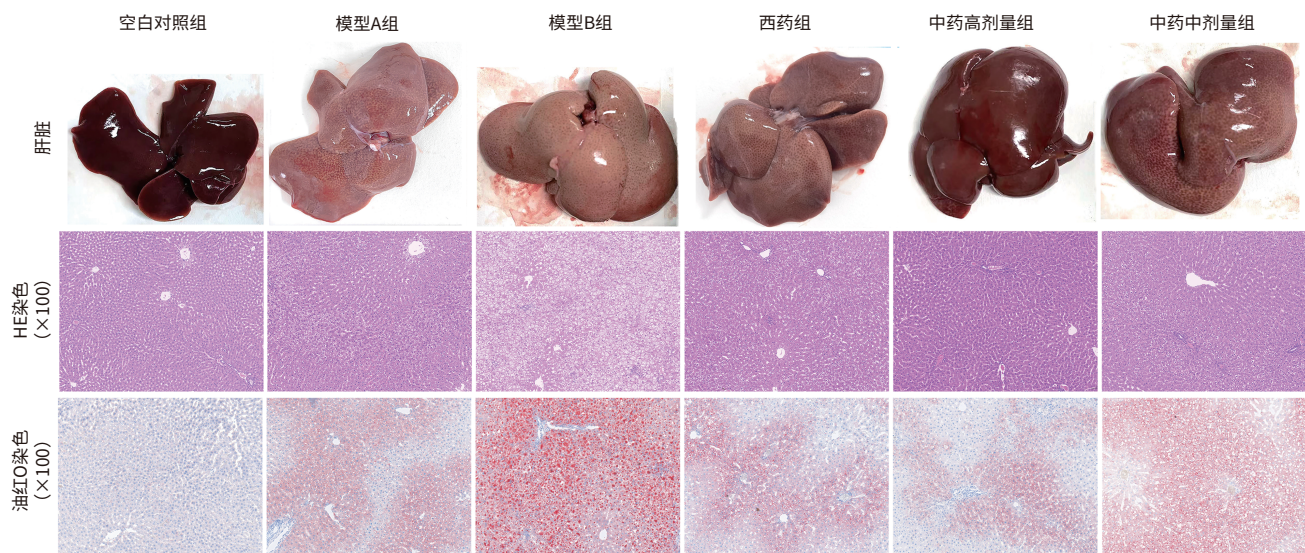


图1 各组大鼠肝组织病理

Figure 1 Pathology of liver tissue in each group of rats

药高剂量组和中药中剂量组的血清sTREM2水平显著降低;中药高剂量组的血清sTREM2水平较西药组、中药中剂量组显著降低(P 值均 <0.05)(表5)。

2.5 化痰祛湿活血方对大鼠巨噬细胞胞葬率的影响
与空白对照组比较,模型A组的巨噬细胞胞葬率显著升高;与模型B组比较,模型A组、西药组、中药高剂量组和

中药中剂量组的巨噬细胞胞葬率显著升高;中药高剂量组的巨噬细胞胞葬率较西药组、中药中剂量组显著升高(P 值均 <0.05)(表6,图2)。上述研究结果提示,化痰祛湿活血方能够提高MASH大鼠巨噬细胞胞葬作用。

2.6 化痰祛湿活血方对大鼠巨噬细胞胞葬相关基因的影响
与空白对照组比较,模型A组TREM2的mRNA水

表3 各组大鼠肝组织染色结果

Table 3 Staining results of liver tissue in each group of rats

| 组别 | 动物数 (只) | 肝细胞脂肪变 | 小叶内炎症 (20倍计数坏死灶) | 肝细胞气球样变 | NAS(分) | 油红O阳性面积 (%) |
|--------|------------|-------------------------------|-------------------------------|-------------------------------|---------------------------|----------------------------|
| 空白对照组 | 5 | 1.00(1.00~1.50) | 0.00(0.00~0.00) | 0.00(0.00~0.50) | 1.40±0.55 | 3.70±0.40 |
| 模型A组 | 5 | 2.00(2.00~3.00) | 0.00(0.00~0.00) ²⁾ | 0.00(0.00~1.00) ²⁾ | 2.80±0.84 ¹⁾²⁾ | 17.44±3.08 ¹⁾²⁾ |
| 模型B组 | 5 | 3.00(3.00~3.00) ¹⁾ | 1.00(1.00~2.00) ¹⁾ | 2.00(1.50~3.00) ¹⁾ | 6.60±1.14 ¹⁾ | 61.91±2.28 ¹⁾ |
| 西药组 | 5 | 2.00(2.00~3.00) | 1.00(1.00~1.50) | 1.00(1.00~2.00) | 5.00±0.71 ²⁾³⁾ | 27.36±1.85 ²⁾³⁾ |
| 中药高剂量组 | 5 | 2.00(1.50~2.00) | 0.00(0.00~1.00) | 1.00(1.50~1.00) | 3.00±1.00 ²⁾ | 18.66±2.18 ²⁾ |
| 中药中剂量组 | 5 | 2.00(1.50~2.50) | 1.00(1.00~1.50) | 1.00(1.00~2.00) | 4.20±1.64 ²⁾ | 29.51±1.63 ²⁾³⁾ |
| 统计值 | | $H=17.690$ | $H=118.964$ | $H=118.495$ | $F=115.538$ | $F=1450.275$ |
| P 值 | | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

注:与空白对照组比较,1) $P<0.05$;与模型B组比较,2) $P<0.05$;与中药高剂量组比较,3) $P<0.05$ 。

表4 各组大鼠血清肝脏酶学、血脂水平

Table 4 Serum liver enzyme and blood lipid levels of rats in each group

| 组别 | 动物数 (只) | ALT (U/L) | AST (U/L) | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | FFA (mmol/L) |
|--------|------------|-----------------------------|-----------------------------|---------------------------|---------------------------|---------------------------|-------------------------|---------------------------|
| 空白对照组 | 10 | 20.24±8.82 | 22.85±7.83 | 2.03±0.22 | 0.77±0.21 | 2.40±0.70 | 0.65±0.21 | 0.94±0.55 |
| 模型A组 | 10 | 26.25±10.07 ²⁾ | 24.63±8.36 ²⁾ | 3.68±0.41 ¹⁾²⁾ | 1.36±0.13 ¹⁾²⁾ | 1.18±0.33 ¹⁾²⁾ | 1.85±0.44 ¹⁾ | 2.38±0.59 ¹⁾²⁾ |
| 模型B组 | 10 | 162.36±37.60 ¹⁾ | 46.11±8.89 ¹⁾ | 6.45±1.45 ¹⁾ | 2.12±0.57 ¹⁾ | 0.58±0.18 ¹⁾ | 1.88±0.73 ¹⁾ | 5.73±2.10 ¹⁾ |
| 西药组 | 10 | 58.07±16.70 ²⁾³⁾ | 34.26±14.57 ²⁾³⁾ | 3.34±0.54 ²⁾ | 1.10±0.37 ²⁾ | 1.28±0.34 ²⁾³⁾ | 1.56±0.81 | 2.36±0.65 ²⁾³⁾ |
| 中药高剂量组 | 10 | 33.70±11.71 ²⁾ | 21.31±6.38 ²⁾ | 2.73±0.48 ²⁾ | 0.87±0.27 ²⁾ | 2.13±0.59 ²⁾ | 1.04±0.29 | 1.14±0.72 ²⁾ |
| 中药中剂量组 | 10 | 59.97±13.12 ²⁾ | 33.41±10.87 ²⁾ | 3.57±0.64 ²⁾ | 1.27±0.51 ²⁾ | 0.95±0.13 ²⁾³⁾ | 1.08±0.38 | 2.52±0.63 ²⁾³⁾ |
| F 值 | | 37.111 | 9.158 | 44.310 | 17.883 | 25.613 | 15.462 | 17.479 |
| P 值 | | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

注:与空白对照组比较,1) $P<0.05$;与模型B组比较,2) $P<0.05$;与中药高剂量组比较,3) $P<0.05$ 。ALT,丙氨酸氨基转移酶;AST,天冬氨酸氨基转移酶;TC,总胆固醇;TG,甘油三酯;HDL-C,高密度脂蛋白胆固醇;LDL-C,低密度脂蛋白胆固醇;FFA,游离脂肪酸。

表5 各组大鼠血清TNF- α 、IL-1 β 、IL-6、sTREM2水平

Table 5 Serum TNF- α , IL-1 β , IL-6 and sTREM2 levels in each group of rats

| 组别 | 动物数 (只) | TNF- α (pg/mL) | IL-1 β (pg/mL) | IL-6 (pg/mL) | sTREM2 (ng/mL) |
|--------|------------|-----------------------------|------------------------------|-----------------------------|----------------------------|
| 空白对照组 | 10 | 229.18±104.00 | 97.03±21.08 | 26.42±6.20 | 7.21±0.71 |
| 模型A组 | 10 | 316.47±45.69 ²⁾ | 203.71±31.49 ¹⁾²⁾ | 57.24±11.69 ¹⁾²⁾ | 8.66±0.60 ¹⁾²⁾ |
| 模型B组 | 10 | 808.13±164.83 ¹⁾ | 355.85±57.04 ¹⁾ | 120.96±40.59 ¹⁾ | 21.13±1.96 ¹⁾ |
| 西药组 | 10 | 601.78±112.25 ³⁾ | 267.74±48.80 ²⁾³⁾ | 61.83±19.49 ²⁾ | 18.52±3.30 ³⁾ |
| 中药高剂量组 | 10 | 371.56±64.32 ²⁾ | 197.96±37.52 ²⁾ | 37.41±11.59 ²⁾ | 11.20±1.44 ²⁾ |
| 中药中剂量组 | 10 | 601.12±231.13 ³⁾ | 249.31±56.64 ²⁾³⁾ | 63.11±36.34 ²⁾³⁾ | 13.71±1.54 ²⁾³⁾ |
| F 值 | | 29.504 | 38.013 | 24.509 | 115.212 |
| P 值 | | <0.01 | <0.01 | <0.01 | <0.01 |

注:与空白对照组比较,1) $P<0.05$;与模型B组比较,2) $P<0.05$;与中药高剂量组比较,3) $P<0.05$ 。TNF- α ,肿瘤坏死因子- α ;IL-1 β ,白细胞介素-1 β ;IL-6,白细胞介素-6;sTREM2,可溶性髓系细胞触发受体2。

平显著升高,模型B组的肝组织S1P、S1PR1和TREM2的mRNA水平显著升高;与模型B组比较,模型A组及各用药组肝组织S1P、S1PR1、ADAM17和TREM2的mRNA水平无统计学差异(P 值均 >0.05)(表7)。

表6 各组大鼠巨噬细胞胞葬率

Table 6 The phagocytic rate of macrophages in each group of rats

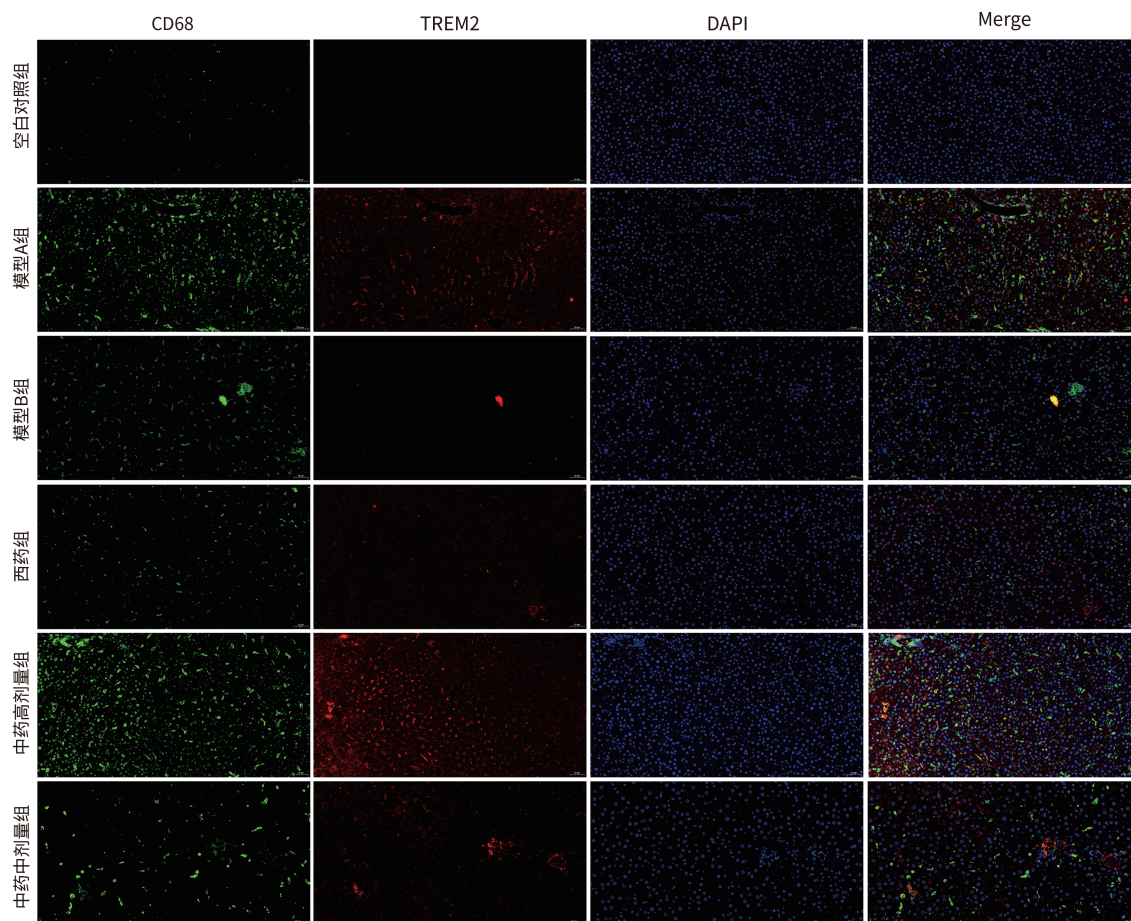
| 组别 | 动物数(只) | 巨噬细胞胞葬率(%) |
|--------|--------|----------------------------|
| 空白对照组 | 5 | 11.55±2.30 |
| 模型A组 | 5 | 51.42±1.90 ¹⁾²⁾ |
| 模型B组 | 5 | 15.14±1.69 |
| 西药组 | 5 | 19.21±2.05 ²⁾³⁾ |
| 中药高剂量组 | 5 | 49.65±5.82 ²⁾ |
| 中药中剂量组 | 5 | 27.07±4.59 ²⁾³⁾ |
| F 值 | | 129.133 |
| P 值 | | <0.01 |

注:与空白对照组比较,1) $P<0.05$;与模型B组比较,2) $P<0.05$;与中药高剂量组比较,3) $P<0.05$ 。

2.7 化痰祛湿活血方对大鼠巨噬细胞胞葬相关蛋白的影响 与空白对照组比较,模型A组、模型B组的肝组织S1P、S1PR1和ADAM17蛋白表达水平显著升高,模型A组的TREM2蛋白表达水平显著升高;与模型B组比较,模型A组的肝组织S1P、S1PR1和ADAM17蛋白表达水平显著降低,TREM2蛋白表达水平显著升高,中药高剂量组、中药中剂量组的ADAM17蛋白表达水平显著降低,中药高剂量组的TREM2蛋白表达水平显著升高(P 值均 <0.05)(表8,图3)。

3 讨论

全国名中医赵文霞教授依据MASH痰瘀互结证的病机创立了化痰祛湿活血方,方中泽泻利水渗湿、化浊降脂为君药;海藻化痰利湿,丹参活血化痰,郁金活血行气,三者共为臣药;山楂化浊散瘀,决明子清肝降脂,水飞蓟清热利湿,三者共为佐药;柴胡疏肝理气为使药,诸药共用,以化痰祛湿活血。现代药理研究显示,该方中



注:TREM2,髓系细胞触发受体2。

图2 各组大鼠巨噬细胞胞葬率结果(免疫荧光,×200)

Figure 2 Phagocytic rate of macrophages in each group of rats (immunofluorescence, ×200)

表7 各组大鼠肝组织S1P、S1PR1、ADAM17、TREM2 mRNA表达水平
Table 7 The mRNA levels of S1P, S1PR1, ADAM17, and TREM2 in liver tissues of rats in each group

| 组别 | 动物数(只) | S1P | S1PR1 | ADAM17 | TREM2 |
|--------|--------|-------------------------|-------------------------|-----------|---------------------------|
| 空白对照组 | 6 | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 |
| 模型A组 | 6 | 2.91±1.13 | 2.60±1.08 ²⁾ | 2.63±1.34 | 2.84±0.60 ¹⁾²⁾ |
| 模型B组 | 6 | 6.73±2.33 ¹⁾ | 7.11±2.98 ¹⁾ | 7.93±3.68 | 7.16±0.93 ¹⁾ |
| 西药组 | 6 | 5.90±0.70 | 6.34±2.47 | 5.61±1.90 | 6.76±1.37 |
| 中药高剂量组 | 6 | 5.94±2.14 | 8.20±7.41 | 3.03±1.04 | 8.50±1.87 |
| 中药中剂量组 | 6 | 5.90±1.86 | 6.70±3.39 | 4.45±1.49 | 6.58±1.42 |
| F值 | | 12.058 | 3.536 | 9.710 | 34.864 |
| P值 | | <0.01 | <0.05 | <0.01 | <0.01 |

注:与空白对照组比较,1)P<0.05;与模型B组比较,2)P<0.05。S1P,鞘氨醇-1-磷酸;S1PR1,鞘氨醇1磷酸酯受体1;ADAM17,解整合素金属蛋白酶17;TREM2,髓系细胞触发受体2。

表8 各组大鼠肝组织S1P、S1PR1、ADAM17、TREM2蛋白表达水平
Table 8 Expression levels of S1P, S1PR1, ADAM17, and TREM2 proteins in liver tissues of rats in each group

| 组别 | 动物数(只) | S1P | S1PR1 | ADAM17 | TREM2 |
|--------|--------|----------------------------|---------------------------|----------------------------|----------------------------|
| 空白对照组 | 5 | 6.24±0.87 | 3.43±0.90 | 5.00±1.01 | 5.24±0.30 |
| 模型A组 | 5 | 11.87±1.77 ¹⁾²⁾ | 8.21±0.78 ¹⁾²⁾ | 13.26±1.72 ¹⁾²⁾ | 34.15±9.15 ¹⁾²⁾ |
| 模型B组 | 5 | 22.47±2.96 ¹⁾ | 35.42±7.86 ¹⁾ | 26.31±1.80 ¹⁾ | 8.52±2.27 |
| 西药组 | 5 | 20.72±1.46 | 30.95±3.53 | 22.54±2.39 ³⁾ | 9.38±2.20 |
| 中药高剂量组 | 5 | 20.99±1.37 | 33.77±5.14 | 15.37±1.32 ²⁾ | 22.74±5.66 ²⁾ |
| 中药中剂量组 | 5 | 22.42±2.57 | 31.25±3.93 | 20.57±1.42 ²⁾³⁾ | 15.02±2.91 |
| F值 | | 59.345 | 50.987 | 129.890 | 27.657 |
| P值 | | <0.01 | <0.01 | <0.01 | <0.01 |

注:与空白对照组比较,1)P<0.05;与模型B组比较,2)P<0.05;与中药高剂量组比较,3)P<0.05。S1P,鞘氨醇-1-磷酸;S1PR1,鞘氨醇1磷酸酯受体1;ADAM17,解整合素金属蛋白酶17;TREM2,髓系细胞触发受体2。

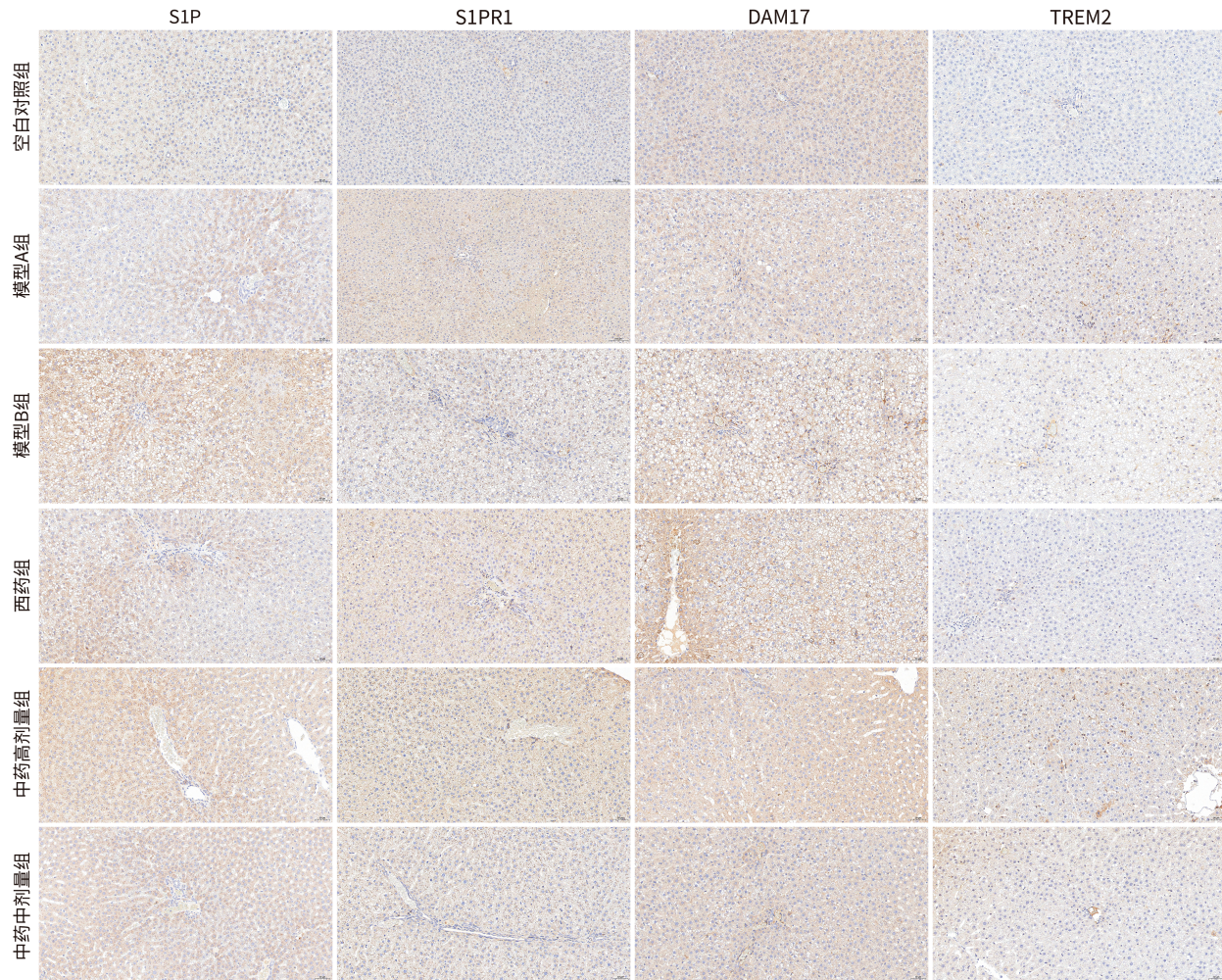
药物具有降脂、保肝和改善肝脏炎症损伤等作用^[11-14]。本研究结果进一步证实,化痰祛湿活血方能够减轻肝脏脂肪变及炎症损伤,与本团队前期研究结论一致^[15]。

胞葬作用是对凋亡细胞清除的过程,对维持正常组织稳态和组织损伤后恢复稳态具有重要作用^[16]。巨噬细胞胞葬作用受损,将导致凋亡细胞在肝内积聚,进而诱发肝组织坏死和炎症^[17]。近期研究表明,巨噬细胞胞葬受体TREM2在MASH发病中发挥关键作用^[18]。在喂食高脂肪/高胆固醇饮食的小鼠中,TREM2的mRNA表达随着MAFLD的进展而增加;但在MASH进展期间,TREM2被裂解为sTREM2,降低单核细胞衍生的肝巨噬细胞上的TREM2蛋白表达,且巨噬细胞TREM2的缺失进一步加速MASH炎症、肝纤维化的发展,并与凋亡肝细胞的增加密切相关^[19]。以上研究表明,肝巨噬细胞上的TREM2可以介导凋亡肝细胞的清除,TREM2裂解可能是MASH进展的重要因素。血清sTREM2可间接反映巨噬细胞上TREM2被裂解程度,血清sTREM2水平越高,说明TREM2被裂解得越多。

MAFL发生时,富含脂质的凋亡肝细胞释放S1P增

加,S1P可激活单核细胞来源的巨噬细胞表面受体S1PR1,进一步促进TREM2的mRNA表达,促使TREM2蛋白表达增多,巨噬细胞通过TREM2识别脂质负载的凋亡肝细胞,进而通过胞葬作用清除凋亡的肝细胞,阻止MASH的发生发展^[9]。然而,长期过度的高营养摄入会导致肝脏中产生的TNF和IL-1 β 等促炎症细胞因子水平上升,进而激活肝脏巨噬细胞中的金属蛋白酶ADAM17,促使TREM2蛋白裂解,导致巨噬细胞无法发挥胞葬作用,不能及时清除富含脂质的凋亡肝细胞,形成恶性循环^[9,20]。因此,阻断巨噬细胞TREM2裂解以恢复巨噬细胞清除凋亡肝细胞的能力,可能是预防和治疗MASH的有效方法。

本研究结果表明,化痰祛湿活血方能够降低MASH大鼠体重、肝湿重和肝指数,改善肝组织病理学所示肝脏脂肪变及炎症损伤,改善血脂(降低TC、TG、LDL-C和FFA,升高HDL-C),降低肝脏酶学指标(ALT、AST),降低血清炎症因子(TNF- α 、IL-1 β 和IL-6)水平和sTREM2水平,且高剂量效果最优,提示化痰祛湿活血方可能通过抑制TREM2蛋白的裂解,改善MASH大鼠肝脏脂肪沉积



注: S1P, 鞘氨醇-1-磷酸; S1PR1, 鞘氨醇1磷酸酯受体1; ADAM17, 解整合素金属蛋白酶17; TREM2, 髓系细胞触发受体2。

图3 各组大鼠巨噬细胞胞葬相关蛋白S1P、S1PR1、ADAM17、TREM2的表达结果(免疫组化, ×200)

Figure 3 Expression of macrophage cell burial related proteins S1P, S1PR1, ADAM17 and TREM2 in each group of rats (immunohistochemistry, ×200)

及炎症损伤。进一步研究表明,化痰祛湿活血方可能通过抑制ADAM17蛋白表达,阻止TREM2蛋白裂解,恢复巨噬细胞胞葬功能,从而发挥治疗MASH的作用。本研究采用CD68与TREM2共定位(CD68⁺TREM2⁺)的方法评估巨噬细胞胞葬率。CD68虽广泛用作巨噬细胞标志物,但也可能在其他髓系细胞中表达;同时,并非所有TREM2阳性细胞均参与胞葬过程。因此,采用CD68与TREM2共定位分析旨在尽可能提高对巨噬细胞胞葬状态评估的特异性,但仍存在一定局限性。未来研究可采用更特异的巨噬细胞胞葬标志物进行验证,以进一步提高结果的准确性。此外,由于中药复方药物多,存在多靶点、多途径治疗疾病的作用,还需深入研究化痰祛湿活血方治疗MASH的其他可能靶点,以完整阐释该方治疗MASH的调控机制。

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