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• 研究快报 BRIEF REPORT •

清热化湿法对温病湿热证大鼠肝脏 LDL-R mRNA 的影响

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Effect of *Qingre Huashi* recipe on liver low density lipoprotein-receptor mRNA in rats with epidemic febrile disease of dampness-heat syndrome

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Abstract

AIM: To establish the rat models of dampness-heat syndrome by stimulation of compound factors, and to investigate the effect of *Qingre Huashi* recipe on the expression of low density lipoprotein-receptor (LDL-R) mRNA in the liver tissues of rats.

METHODS: Forty-eight male Wistar rats were randomly divided into six groups: normal control, dampness-heat syndrome model, *Qingre Huashi* (A), *Qingre Jiedu* (B), *Xuanqi Huashi* (C), and Lipanthyl (D) group. Each group contained 8 rats. Except the rats in the normal control group, the ones in other groups were all treated with the fat and sweet diet plus artificial climate box plus Salmonella typhimurtum to establish the model of dampness-heat syndrome. Then the rats in A, B, C, D group were treated the corresponding medicine for 5 d. The expression of LDL-R mRNA was detected in the liver tissues of rats by Using reverse transcription polymerase chain reaction.

RESULTS: The expression of LDL-R mRNA was significantly lower in model group than that in the normal

controls (*P* = 0.005), while the expression of LDL-R mRNA was markedly higher in A, B, C and D group than that in the model group (0.779 1±0.042 5, 0.616 1±0.038 4, 0.631 5±0.028 3, 0.800 4±0.039 4 *vs* 0.473 8±0.048 0, all *P* <0.01). LDL-R mRNA expression was notably different between group A and B (*P* = 0.04), but not between group A and C (*P* >0.05), B and C (*P* >0.05), as well as A and D (*P* >0.05).

CONCLUSION: *Qingre Huashi* recipe can increase the expression of LDL-R mRNA in the liver tissues of rat model of dampness-heat syndrome.

Key Words: *Qingre Huashi* recipe; Epidemic febrile disease; Dampness-heat syndrome; Low density lipoprotein-receptor mRNA; Rats

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摘要

目的: 采用复合因素造模法建立大鼠湿热证模型, 探讨清热化湿法(复合治法)对该湿热证模型大鼠肝脏LDL-R mRNA表达的影响.

方法: 清洁级Wistar♂大鼠48只, 随机分成正常对照组、湿热证模型组、清热化湿(A)法组、清热解毒(B)法组、宣气化湿(C)法组和力平脂(D)组, 每组8只. 除正常对照组外, 其余组以“肥甘饮食+人工气候箱+鼠伤寒沙门菌”法造模. 完成后治疗组连续给药5 d. 模型组给予等量生理盐水. 采用逆转录-聚合酶链反应(RT-PCR)技术检测LDL-R mRNA.

结果: 模型组大鼠肝脏LDL-R mRNA的表达显著低于正常对照组(*P* = 0.005). A、B、C、D组LDL-R mRNA的表达显著高于模型组(0.779 1±0.042 5, 0.616 1±0.038 4, 0.631 5±0.028 3, 0.800 4±0.039 4 *vs* 0.473 8±0.048 0, 均*P*<0.01). A 组升高LDL-R mRNA优于B组(*P* = 0.04); A组与C组相比无显著性差异(*P*>0.05); B组与C组比较, 两者无显著性差异(*P*>0.05); A组与D组比较无显著性差异(*P*>0.05).

结论: 清热化湿法可增强湿热证模型大鼠肝脏LDL-R mRNA表达.

关键词: **清热化湿法**; **温病湿热证**; LDL–R mRNA; **大鼠**

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0 引言

清热化湿法可明显降低湿热证模型大鼠LDL-C含量, 促进肝脏中的LDL-R表达, 发挥其调节体内胆固醇平衡的作用[1-3]. LDL与LDL-R结合后才能发挥效应, 而LDL-R mRNA的表达则对LDL-R的表达起着调控作用. 为从更深层次揭示清热化湿法调节脂质代谢的作用机制, 我们应用逆转录-聚合酶链反应技术, 从基因水平观察清热化湿法对LDL-R mRNA表达的影响.

1 材料和方法

1.1 材料 清洁级Wistar♂大鼠48只, 体质量200±20 g, 由华中科技大学同济医学院实验动物学部提供, 清热化湿(A)法: 黄连20 g, 厚朴^{另包}20 g, 藿香^{另包}20 g, 石菖蒲^{另包}20 g, 丹参16 g, 土大黄10 g, 茯苓20 g, 栀子10 g; 清热解毒(B)法: 黄连20 g, 黄芩15 g, 黄柏15 g, 栀子20 g; 宣气化湿(C)法: 杏仁18 g, 薏苡仁18 g, 蔻仁6 g, 竹叶6 g, 厚朴6 g, 通草6 g, 半夏15 g, 滑石18 g; 力平脂(D)组: 力平脂(法国利博福尼制药公司生产), 使用前用生理盐水配成1.8 g/L混悬液. TRIzol Reagent Total RNA试剂盒购自美国Invitrogen公司; RNA PCR试剂盒[RNA PCR Kit (AMV) Ver3.0]购自大连宝生生物有限公司. 丙烯酰胺和定标用的蛋白为Sigma公司产品, 标准分子质量DNA marker购自日本TaKaRa公司.

1.2 方法 大鼠随机分成正常对照组、湿热证模型组、A、B、C、D组, 每组8只. 除空白对照组外, 其余模型组、治疗A、B、C、D组以“肥甘饮食+人工气候箱+鼠伤寒沙门氏菌”法造模[4]; 维持5-7 d左右, 造模成功. 治疗组每天给药1次, 连续5 d. 模型组给予等量生理盐水, 第5天给药前禁食12 h, 给药60 min后, 用30 g/L戊巴比妥钠30-50 mg/kg麻醉, 切开腹部迅速取肝脏, 液氮冻存供LDL-R mRNA检测; 用TRIzol核酸提取试剂提取肝细胞总RNA测定RNA样品含量及纯度: A_{260}/A_{280} = 1.8-2.0, 说明其纯度满足分子生物学实验的要求. 在NCBI上查找大鼠LDL-R基因序列, 然后用Primer Primier5软件设计引物, 并由北京奥科生物技术有限责任公司合成. 内对照为3磷酸甘油醛脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, GAPDH). LDL-R引物序列: (GenBank No.M94388)上游引物: 5’-AAC TAG ACT GCTCCC CCA AGA C -3’, 下游引物: 5’-CTG CGA TGGATA CAC TCACTA CTG-3’, 扩增的cDNA序列片段长度为340 bp. GAPDH引物序列: 上游引物: 5’-CCA TGG AGA AGG CTG GGG-3’, 下游引物: 5’-CAA AGT TGT CAT GGA TGA CC-3’, 扩增的cDNA序列片段长度为309 bp.

在PCR仪中设置逆转录程序: 30℃×10 min-42℃×30 min-99℃×5 min-4℃×5 min. 每一管反应前液面上需加矿物油50 μL, 防止高温下的挥发. 反应停止后置于4℃备用. 在PCR仪中设置PCR程序: 94℃×4 min-(94℃×45 s-45℃×45 s-72℃×1 min)×35个循环-72℃×5 min-4℃保存. 反应停止后, 取PCR反应产物10 μL, 进

行20/L琼脂糖凝胶电泳(含0.5 mg/L溴化乙锭)分析, 经凝胶密度扫描系统处理, 测定各电泳带灰度值. 各反应体系以GAPDH mRNA的RT-PCR产物为内参照, 计算待测mRNA的RT-PCR产物与它的灰度比值, 作为待测mRNA的表达值. LDL-R mRNA相对量 = LDL-R电泳带灰度值/GAPDH电泳带灰度值.

统计学处理 用SPSS 10.0统计软件, 数据以mean±SD表示, 采用单因素方差分析, 组间用q检验, P<0.05为有显著性差异.

2 结果

肝细胞总RNA提取结果见图1. 正常对照组、湿热模型组、治疗A、B、C、D组大鼠肝脏LDL-R mRNA表达分别为0.882 0±0.058 5, 0.473 8±0.048 0, 0.779 1±0.042 5, 0.616 1±0.038 4, 0.631 5±0.028 3, 0.800 4±0.039 4(图2). 模型组大鼠肝脏LDL-R mRNA的表达显著低于正常对照组(P = 0.005<0.01); A、B、C、D组LDL-R mRNA的表达显著高于模型组(P = 0.001<0.01). 以A法与B法比较, A法升高LDL-R mRNA优于B法(P = 0.04<0.05); 以A法与C法比较, 虽然无显著性差异, 但A法LDL-R mRNA相对含量高于C组; 以B法与C法比较, 两者无显著性差异(P>0.05); 以A法与力平脂组比较无显著性差异(P>0.05), 提示清热化湿(A)法具有上调LDL-R mRNA的作用, 与力平脂的作用相当.

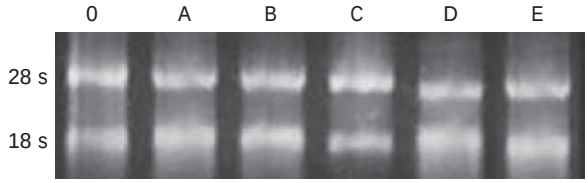

图1 Total RNA甲醛胶电泳.

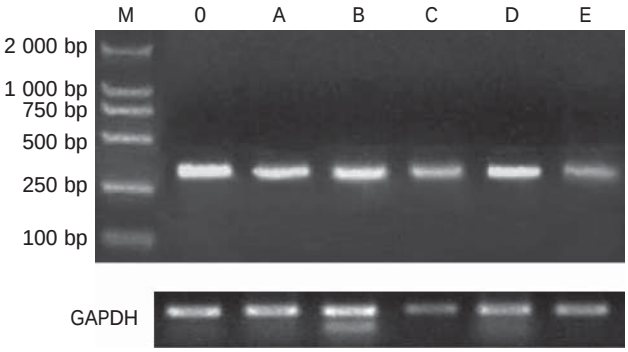

图2 PCR的结果电泳图. M: marker; 0: 正常对照组; A: 清热化湿法组; B: 清热解毒法组; C: 宣气化湿法组; D: 力平脂组; E: 模型组.

3 讨论

脂类代谢异常为湿邪生化物质基础之一, 清热化湿法作用机制与调控脂类代谢相关[1,2], 湿热证动物模型稳定, 可重复性好[3-7]. 本课题组在此基础上研究了清热化湿法对湿热模型大鼠脂类代谢的影响, 表明该法可明显降低LDL-C、升高HDL-C; 肝脏是人体脂蛋白及胆固醇代谢的主要场所, 也是LDL-R较为密集的器官. 肝脏LDL-R表达的变化可反映血清胆固醇的代谢[8,9]. 肥甘饮食中的胆固醇降低了肝脏LDL-R mRNA水平, 因此降低了肝脏LDL-R数量, 引起血循环中LDL的堆积[10].

LDL-R功能缺陷, 是引起高胆固醇血症的主要原因之一, 血脂失调症患者的LDL-R转录水平处于严重的抑制状态[11]. 中医药领域有关调节脂类代谢的作用机制较多[12-15]. 但是对LDL受体研究较少, 研究[16,17]证实, 中药亦可提高LDL-R基因表达, 解除LDL-R的抑制状态, 促进LDL-R表达, 使血中LDL-C减少, 从而调控血脂水平. 本结果表明, 温病湿热证模型组LDL-R mRNA表达明显低于正常组, 提示湿热证病理状态下, 降低了肝脏LDL-R mRNA水平, 抑制了LDL-R的转录, 降低了肝脏LDL-R数量, 引起血循环中LDL的堆积, 此与有关文献报道的观点相似[18-20]. 研究显示: 治疗各组LDL-R mRNA表达均高于湿热模型组, 表明各治疗组药物通过提高LDL-R mRNA的表达水平, 降低湿热证对LDL-R转录的抑制作用, 比较清热化湿(A)法与清热解毒(B)法、宣气化湿(C)法升高LDL-R mRNA表达情况, A法优于B、C法.

总之, 清热化湿法可能通过增强湿热证模型大鼠肝脏LDL-R mRNA表达, 继而促进LDL-R蛋白合成, 增加了肝表面的LDL-R数量与活性, 增强肝脏对脂质等代谢功能, 加快血中LDL-C的清除, 加速脂蛋白分解, 从而起到了调控血脂水平的作用. 这可能是清热化湿法调节脂类代谢的作用机制之一.

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• 研究快报 BRIEF REPORT •

熊胆粉对二甲基亚硝胺诱发大鼠肝纤维化的抑制作用

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Inhibitory effects of bear bile powder on dimethylnitrosamine-induced liver fibrosis in rats

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Abstract

AIM: To investigate the inhibitory effect of bear bile powder on rat liver fibrosis induced by dimethylnitrosamine (DMN).

METHODS: A total of 30 rats were randomly divided into 3 groups: normal control group, model group, and bear bile group (10 in every group). The rat liver fibrosis model was induced by peritoneal injection of DMN (10 g/L) for 4 wk. At the same time, the rats in the bear bile group received bear bile powder (400 mg/kg) orally once a day for 4 wk. Then the activities of serum alanine transaminase (ALT), aspartate transaminase (AST), and the contents of total protein (TP) were detected. Meanwhile, the pathological changes of liver tissues were observed under light microscope after HE staining, and the area density of collagenous fiber were examined. The amount and distribution of Kupffer cell (KC) and hepatic stellate cell (HSC) were detected by immunohistochemical SP method through the distribution of ED1 and α-smooth muscle actin (α-SMA).

RESULTS: Compared with that in the model group, the level of serum ALT was decreased, and AST was significantly decreased (4 370.87±1 338.60 nkat/L *vs* 5 741.15±1 000.20 nkat/L, P <0.05) in the bear bile group. The TP content and the ratio of liver to body weigh were increased. The area density of collagenous fiber was notably decreased (6.73±1.31 *vs* 9.90±1.93, P <0.01). The pathological changes were lighter, and the occurrence rate of diffused liver fibrosis was lower in the bear bile group than those in the model group. The fibrous septa became thin or disappeared, and the expressions of ED1 and α-SMA were markedly reduced in the bear bile group.