· 论 著 ·

奥贝胆酸联合白藜芦醇对小鼠非酒精性脂肪性肝病的治疗作用

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摘要: 目的 探索奥贝胆酸(OCA)和白藜芦醇(RSV)联合应用对非酒精性脂肪性肝病(NAFLD)模型小鼠的治疗作用和机制。**方法** 雄性C57BL/6N小鼠42只,除正常对照组8只外,其余用1%四氯化碳(CCl_4)按5 mL·kg^{-1}每周ip注射1次联合高脂饲料喂食4周诱导NAFLD模型。建模小鼠分为模型对照组、模型+OCA组、模型+RSV组和模型+OCA+RSV组,OCA和RSV均30 mg·kg^{-1} ig给药连续28 d。小鼠处死取血制备血清,取肝称重计算肝指数, HE染色观察肝组织病理形态,油红O染色检测肝细胞脂质沉积并统计脂滴面积百分比;全自动生化分析仪和检测试剂盒检测血清谷丙转氨酶(GPT)、谷草转氨酶(GOT)、总胆固醇(TC)、甘油三酯(TG)、低密度脂蛋白(LDL)、高密度脂蛋白(HDL)、超氧化物歧化酶(SOD)活性、丙二醛(MDA)、白细胞介素1β(IL-1β)、IL-6和肿瘤坏死因子α(TNF-α)水平;Western印迹法检测肝组织沉默信息调节因子1(Sirt1)、NF-κB和p-NF-κB蛋白表达水平。**结果** 与正常对照组相比,模型组小鼠肝指数显著升高(P<0.01);与模型组相比,各给药组肝指数显著降低(P<0.01);与模型+RSV相比,模型+OCA+RSV组肝指数降低更加明显(P<0.05)。与正常对照组相比,模型组小鼠肝组织有大量脂肪空泡和炎症损伤,油红O染色后细胞内有大量红色脂滴,血清GPT,GOT,TC,LDL,MDA,IL-1β,IL-6和TNF-α水平及肝组织NF-κB蛋白表达和磷酸化水平显著升高(P<0.01),血清HDL水平和肝组织Sirt1表达水平显著降低(P<0.05)。与模型组相比,模型+OCA组肝细胞形态正常,肝组织少量脂肪变性空泡,红色脂滴减少,血清GOT,TC,MDA,IL-6和TNF-α水平显著降低(P<0.05),GTP,HDL和LDL水平无明显变化;模型+RSV组脂滴空泡无明显减少,细胞内红色脂滴略有减少,血清TC,LDL,MDA,IL-1β,IL-6和TNF-α水平显著降低(P<0.05),GPT,GOT和HDL水平无明显变化,肝组织p-NF-κB蛋白表达水平显著降低(P<0.05),肝组织Sirt1表达水平显著升高(P<0.05);模型+OCA+RSV组脂滴空泡显著减少,细胞内脂质沉积显著减轻,血清GPT,GOT,TC,LDL,MDA,IL-1β,IL-6和TNF-α水平及肝组织NF-κB和p-NF-κB蛋白表达水平显著降低(P<0.01),血清HDL水平和肝组织Sirt1表达水平显著升高(P<0.01)。与模型+OCA组相比,模型+OCA+RSV组GPT,TC,LDL,MDA,IL-1β和IL-6水平及p-NF-κB表达水平显著降低(P<0.05),血清HDL水平显著升高(P<0.01)。与模型+RSV组相比,模型+OCA+RSV组肝细胞脂质沉积减少,血清GPT,GOT和MDA水平显著降低(P<0.05),HDL水平显著升高(P<0.05)。**结论** OCA与RSV联用可增加抗氧化应激能力,并通过调节Sirt1和NF-κB蛋白表达抑制炎症反应,从而最终发挥对小鼠NAFLD的治疗作用。

关键词: 奥贝胆酸; 非酒精性脂肪肝; 白藜芦醇; 氧化应激
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非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD)包括单纯性脂肪变性、非酒精性脂肪性肝炎(non-alcoholic steatohepatitis, NASH)和肝硬化,严重者可发展为肝细胞癌[1]。目前全球25%的人患有NAFLD[2],并随生活水平的提高NAFLD患病率持续上升[3-4]。奥贝胆酸(obeticholic acid, OCA)是一种法尼醇X受体(farnesoid X receptor, FXR),能够调节脂质和葡萄糖代谢的转录因子,主要作用是通过抑制编码胆固醇7α-羟化酶(cholesterol 7α-hydroxylase, CYP7A1)的细胞

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色素 P450 基因表达和提高胆汁酸转运蛋白表达而减少胆汁酸[5]。此外，OCA 可有效降低 NAFLD 患者脂肪变性，改善肝组织纤维化[6]，但其低密度脂蛋白（low-density lipoprotein，LDL）和总胆固醇（total cholesterol，TC）升高，高密度脂蛋白（high-density lipoprotein，HDL）降低，增加心血管疾病风险等问题较为突出[7-8]。白藜芦醇（resveratrol，RSV）是一种抗衰老、抗氧化、抗炎和胰岛素敏感的天然多酚化合物，对过氧化氢诱导的细胞凋亡有抑制作用[9]，可缓解炎症刺激、氧化应激及细胞凋亡等产生的肝损伤[10]。另外，可通过调节脂代谢升高 HDL、降低 LDL 而降低心血管疾病风险[11]，还可降低肌酸激酶（creatine kinase，CK）、乳酸脱氢酶（lactate dehydrogenase，LDH）和丙二醛（malondialdehyde，MDA）水平，增加超氧化物歧化酶（superoxide dismutase，SOD）活性，减少心肌梗死面积而发挥对心肌梗死的治疗作用[12]。本研究旨在探索 OCA 与 RSV 联用对 NAFLD 的治疗作用及其可能的作用机制，以期为 OCA 联合 RSV 治疗 NAFLD 提供实验依据。

1 材料与方法

1.1 药物、试剂和主要仪器

OCA，纯度 98%，厦门海乐景生化有限公司；白藜芦醇，纯度 98% 上海迈瑞尔化学技术有限公司；SOD 和 MDA 测试盒，南京建成生物工程研究所；谷丙转氨酶（glutamic oxaloacetic transaminase，GOT）、谷草转氨酶（glutamic pyruvic transaminase，GPT）、甘油三酯（triglycerides，TG）、TC、HDL 和 LDL 检测试剂盒，美国贝克曼库尔特有限公司；白细胞介素 1β（interleukin-1β，IL-1β）、IL-6 和肿瘤坏死因子 α（tumor necrosis factor-α，TNF-α）检测试剂盒，上海炫雅生物科技有限公司；兔抗小鼠沉默信息调节因子 1（sirtuin 1，Sirt1）、NF-κB 和磷酸化 NF-κB（p-NF-κB）单克隆抗体（一抗）及 IRDye 800CW 荧光标记山羊抗兔 IgG 抗体（二抗），美国 Cell Signaling Technology 公司；紫外可见分光光度计（型号 UV-26001），津岛仪器（苏州）有限公司；全自动生化分析仪（型号 AU5800），美国贝克曼库尔特有限公司；多功能微孔板读板机（型号 Spectra Max i3x），美国 Molecular Devices 公司。

1.2 动物、NAFLD 模型制备和分组

42 只 SPF 级雄性 C57BL/6N 小鼠〔许可证号：SCXK（京）2016-0006〕，6 周龄，体重 16~18 g，北京维通利华实验动物技术有限公司。小鼠自由饮水和进食，定期更换垫料，在环境安静、温度 23~25℃、相对湿度 40%～60% 和 12 h 昼夜交替条件下饲养。小鼠随机分为正常对照组（8 只）和建模组（34 只），建模小鼠每周 1 次 ip 给予 1% 四氯化碳（CCl_4）5 mL·kg^{-1}，同时饲喂高脂饲料（由 15% 猪油、2.8% 胆固醇、0.28% 甲硫氧嘧啶、0.7% 胆酸钠和 81.2% 普通饲料组成），持续 28 d。随机选取 2 只小鼠，处死后观察肝体积略增大，包膜紧张光滑，边缘变钝，质地柔软，色黄，有油腻感；切片观察有大量脂滴视为 NAFLD 模型制备成功。将建模小鼠随机分为模型组、模型+RSV 组、模型+OCA 组和模型+OCA+RSV 组，OCA 和 RSV 均 30 mg·kg^{-1}（参考文献[13]），每组 8 只；每天 ig 给药 1 次，连续给药 28 d；正常对照组和模型组 ig 给予等体积 0.5% 羧甲纤维素钠混悬液。

1.3 样本制备

给药 28 d 后，禁食不禁水 12 h，次日对各组小鼠称量体重，处死后取血于 1.5 mL 离心管中，制备血清，−20℃冰箱中保存。取肝并称重，于肝左叶切取 2 块 5 mm×5 mm×5 mm 肝组织，4% 多聚甲醛固定，其余−80℃冻存。

1.4 计算肝指数

据 **1.3** 体重和肝重计算肝指数。肝指数=肝重（g）/体重（g）×100。

1.5 HE 染色观察肝组织病理变化

取 **1.3** 制备的肝组织，经石蜡固定、HE 染色后于 200 倍镜下观察炎症细胞浸润、脂肪空泡、纤维化和组织结构的变化。

1.6 油红 O 染色观察肝组织脂质沉积和脂滴面积

取 **1.3** 制备的肝组织，OCT 包埋，冰冻切片，进行油红 O 染色，吸水纸吸干水分后甘油明胶封片。油红 O 染色后脂肪呈红色，正常肝组织显示青蓝色。于显微镜镜下（×200）观察肝细胞内脂肪变化，并用图像分析软件 Image-Pro Plus 6.0 定量分析脂滴面积。脂滴面积百分比（%）=红色脂滴覆盖面积/总测量面积×100%。

1.7 全自动生化仪检测血清 GPT，GOT，TG，TC，HDL 和 LDL 水平

取 **1.3** 制备的血清，用全自动生化仪检测小鼠血清 GPT，GOT，TG，TC，HDL 和 LDL 水平。

1.8 检测血清 SOD 活性和 MDA 含量

取 **1.3** 制备的小鼠血清，按试剂盒说明书检测血清 SOD 活性和 MDA 含量。用紫外分光光度仪分别于 550 和 532 nm 波长检测各孔吸光度值。

1.9 ELISA检测血清IL-1β,IL-6和TNF-α含量

取**1.3**制备的血清,用ELISA试剂盒检测血清IL-1β,IL-6和TNF-α含量。

1.10 Western印迹法检测肝组织Sirt1,NF-κB和p-NF-κB蛋白水平

取**1.3**冻存肝组织,各组称取50~100 mg提取总蛋白,用BCA法进行蛋白定量。取等量蛋白,加入等体积上样缓冲液,混匀后99℃恒温金属浴10 min。每孔上样15 μL,80 V,电泳30 min,再用120 V电压电泳100 min。冰浴,200 mA电流,转膜100 mim。按1∶500稀释一抗,4℃孵育过夜;按1∶5000稀释二抗,室温孵育120 min。以目标蛋白和内参蛋白积分吸光度值比值表示目标蛋白相对表达水平。

1.11 统计学分析

实验结果数据用$\bar{x}\pm s$表示,使用统计软件GraphPad Prism 8用单因素方差分析和*t*检验进行统计学分析。*P*<0.05认为差异具有统计学意义。

2 结果

2.1 奥贝胆酸与白藜芦醇联用对NAFLD模型小鼠肝指数的影响

表1结果显示,与正常对照组相比,模型组小鼠肝指数明显升高(*P*<0.01)。与模型组相比,模型+OCA组、模型+RSV组和模型+OCA+RSV组小鼠肝指数明显降低(*P*<0.01)。与模型+RSV组相比,模型+OCA+RSV组肝指数明显降低(*P*<0.05),但与模型+OCA相比,其肝指数降低不明显。表明OCA与RSV联用比RSV单用可更有效降低肝指数。

Tab.1 Effect of obeticholic acid (OCA) combined with resveratrol (RSV) on liver indexes of model mice with non-alcoholic fatty liver disease (NAFLD)

Group	Liver index
Normal control	2.8±0.6
Model	5.4±0.6**
Model+OCA	4.0±0.4##
Model+RSV	4.4±0.4##
Model+OCA+RSV	3.3±0.6##△

Mice were ip given 1% CCl_4 per weekly at 5 mL·kg^{-1} and fed a high-fat feed (consisting of lard 15%, cholesterol 2.8%, methylthiopyrimidine 0.28%, sodium cholate 0.7%, and ordinary feed 81.2%) for 4 weeks, and the NAFLD model was prepared. The mice were ig administered OCA 30 mg·kg^{-1} or (and) RSV 30 mg·kg^{-1} once a day for 28 d, respectively. Liver index=liver mass (g)/body mass (g)×100. $\bar{x}\pm s$, *n*=8. **P*<0.01, compared with normal control group; ##*P*<0.01, compared with model group; △ *P*<0.05, compared with model+RSV group.

2.2 奥贝胆酸与白藜芦醇联用对NAFLD模型小鼠肝组织病理变化的影响

HE染色结果如图1显示,正常对照组小鼠肝小叶结构清晰,肝细胞排列整齐,无炎症细胞浸润,细胞无脂变。模型组肝小叶界限不清,肝细胞索排列紊乱,出现弥漫性脂肪变性,可见大小不等、数量不一的脂滴空泡。模型+OCA组和模型+RSV组肝小叶不清晰,肝细胞形态大致正常且大小较一致,部分肝细胞轻度水样变性,脂滴空泡减少。模型+OCA+RSV组肝小叶清晰,肝细胞大小较一致且形态正常,脂滴空泡减少,脂质沉积程度减轻。

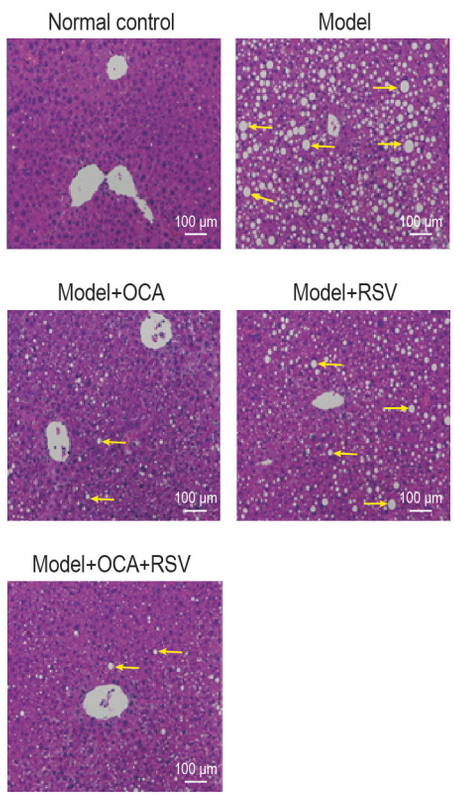

Fig.1 Effect of OCA combined with RSV on morphogolical changes in liver tissue of model mice with NAFLD (HE staining). See Tab. 1 for the mouse treatment. Arrows show fat droplets form vacuoles, the greater the number, the greater the fat content.

2.3 奥贝胆酸与白藜芦醇联用对NAFLD模型小鼠肝细胞中脂质沉积的影响

如图2A所示,正常对照组小鼠肝细胞几乎无红染,无脂变。模型组小鼠肝细胞内脂滴被染成红色,大小不一,广泛分布在胞质中,提示大量肝细胞内存在脂质沉积。模型+OCA组和模型+RSV组小鼠肝细胞内红色脂滴减少,脂质沉积程度有所减

轻。模型+OCA+RSV组肝细胞内红色脂滴减少，脂质沉积程度减轻。如图2B所示，与正常对照组相比，模型组脂滴面积百分比明显升高（P<0.01）。

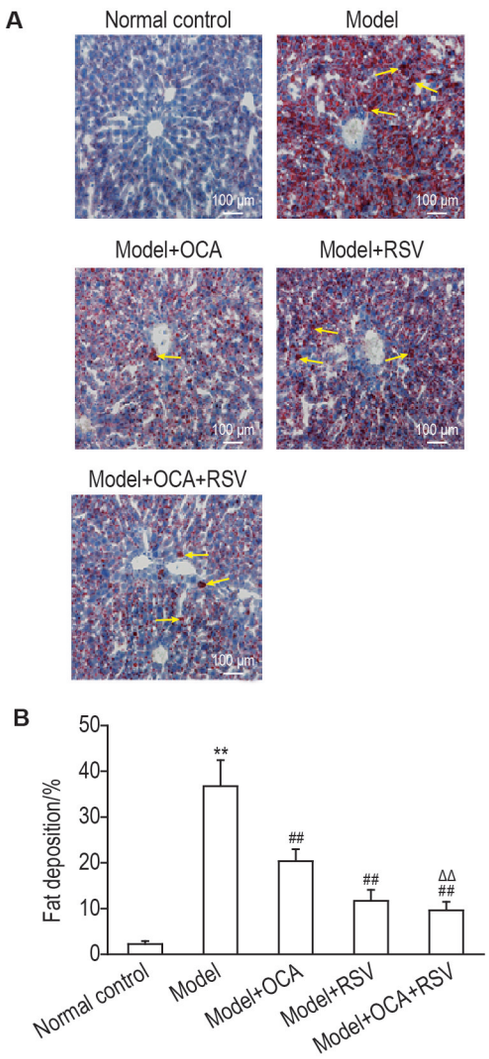

Fig.2 Effect of OCA combined with RSV on hepatocyte fat deposition in model mice with NAFLD by oil red O staining. See Tab.1 for the mouse treatment. Arrows show fat droplets stained with oil red O to form red areas. $\bar{x}\pm s$, n=8. **P<0.01, compared with normal control group; ##P<0.01 compared with model group; △P<0.05, △△P<0.01, compared with model+RSV group.

与模型组相比，模型+OCA组、模型+RSV组和模型+OCA+RSV组脂滴面积百分比明显降低（P<0.01）。与模型+RSV组相比，模型+OCA+RSV组脂滴面积百分比明显减低（P<0.01）。

2.4 奥贝胆酸与白藜芦醇联用对NAFLD模型小鼠肝功能和血脂的影响

由表2可知，与正常对照组相比，模型组血清GPT，GOT，TG，TC和LDL水平明显升高，HDL水平明显降低（P<0.01），提示模型小鼠发生肝损伤和血脂紊乱。与模型组相比，模型+OCA组血清GPT，HDL和LDL水平变化均不明显，GOT，TG和TC水平明显下降（P<0.05）；模型+RSV组血清GPT，GOT和HDL水平变化不明显，TG，TC和LDL水平明显下降（P<0.01）；模型+OCA+RSV组血清GPT，GOT，TG，TC和LDL水平均明显下降（P<0.01），HDL水平明显升高（P<0.01）。与模型+OCA组相比，模型+OCA+RSV组GPT，TC和LDL水平明显降低（P<0.05，P<0.01），HDL水平明显升高（P<0.01）。与模型+RSV组相比，模型+OCA+RSV组GPT和GOT水平明显降低（P<0.05，P<0.01），HDL水平明显升高（P<0.05）。

2.5 奥贝胆酸与白藜芦醇联用对NAFLD模型小鼠血清SOD活性和MDA含量的影响

由表3可知，与正常对照组相比，模型组小鼠血清SOD活性明显降低，MDA含量明显升高（P<0.01），提示模型小鼠肝组织发生氧化应激。与模型组相比，模型+OCA组SOD活性明显升高（P<0.01），MDA含量下降（P<0.05）；模型+RSV组SOD活性明显升高（P<0.01）；模型+OCA+RSV组SOD活性明显升高，MDA含量明显下降（P<0.01）。与模型+OCA组和模型+RSV组相比，模型+OCA+RSV组SOD活性变化不明显，但MDA含量明显降低（P<0.01）。表明两者联合用药比两者单用具有更好的抗氧化应激作用。

Tab.2 Effect of OCA combined with RSV on levels of serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) of model mice with NAFLD

Group	GPT/U·L^{-1}	GOT/U·L^{-1}	TG/mmol·L^{-1}	TC/mmol·L^{-1}	HDL/mmol·L^{-1}	LDL/mmol·L^{-1}
Normal control	36±6	132±12	1.54±0.18	3.15±0.30	2.30±0.11	0.56±0.10
Model	212±15**	252±29**	2.32±0.47**	3.94±0.51**	1.51±0.26**	0.95±0.09**
Model+OCA	171±43	169±21#	1.30±0.40##	3.23±0.37#	1.43±0.45	0.76±0.11
Model+RSV	182±10	209±42	1.62±0.65##	2.13±0.60##	1.52±0.27	0.57±0.12##
Model+RSV+OCA	100±43##△△▲▲	165±53##▲	1.26±0.47##	1.63±0.41##△△	1.88±0.23##△△▲	0.41±0.15##△

See Tab.1 for the mouse treatment. $\bar{x}\pm s$, n=8. **P<0.01, compared with normal control group; #P<0.05, ##P<0.01, compared with model group; △P<0.05, △△P<0.01, compared with model+ OCA group; ▲P<0.05, ▲▲P<0.01, compared with model+ RSV group.

Tab.3 Effects of OCA combined with RSV on serum superoxide dismutase (SOD) activity and malondialdehyde (MDA) content of model mice with NAFLD

Group	SOD/kU·L^{-1}	MDA/μmol·L^{-1}
Normal control	330±12	0.56±0.10
Model	279±46**	10.86±0.33**
Model+OCA	331±26$^{\#\#}$	8.13±0.84$^{\#}$
Model+RSV	347±18$^{\#\#}$	8.33±1.19
Model+OCA+RSV	342±24$^{\#\#}$	6.32±0.80$^{\#\#\triangle\triangle\blacktriangle\blacktriangle}$

See Tab.1 for the mouse treatment. $\bar{x}\pm s$, n=8. **P<0.01, compared with normal control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with model group; $^{\triangle\triangle}P$<0.01, compared with model+OCA group; $^{\blacktriangle\blacktriangle}P$<0.01, compared with model+RSV group.

2.6 奥贝胆酸与白藜芦醇联用对NAFLD模型小鼠血清IL-1β,IL-6和TNF-α水平的影响

由表4可知,与正常对照组相比,模型组小鼠血清IL-1β,IL-6和TNF-α水平明显增加(P<0.01)。与模型组相比,模型+OCA组IL-1β水平变化不显著,IL-6和TNF-α水平明显降低(P<0.05,P<0.01);模型+RSV组IL-1β,IL-6和TNF-α水平明显降低(P<0.01);模型+OCA+RSV组IL-1β,IL-6和TNF-α水平均明显降低(P<0.01)。与模型+RSV组相比,模型+OCA+RSV组IL-6水平明显降低(P<0.01)。与模型+OCA组相比,模型+OCA+RSV组IL-1β和IL-6明显降低(P<0.01)。

Tab.4 Effect of OCA combined with RSV on serum interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) levels of model mice with NAFLD

Group	IL-1β/ng·L^{-1}	IL-6/ng·L^{-1}	TNF-α/ng·L^{-1}
Normal control	22.5±2.5	49±7	112±22
Model	33.3±1.1**	84±5**	259±41**
Model+OCA	29.8±1.5	76±3$^{\#}$	167±16$^{\#\#}$
Model+RSV	26.5±4.4$^{\#\#}$	68±3$^{\#\#}$	175±15$^{\#\#}$
Model+OCA+RSV	24.5±2.4$^{\#\#\triangle\triangle}$	53±5$^{\#\#\triangle\triangle\blacktriangle\blacktriangle}$	170±36$^{\#\#}$

See Tab.1 for the mouse treatment. $\bar{x}\pm s$, n=8. **P<0.01, compared with normal control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with model group; $^{\triangle\triangle}P$<0.01, compared with model+OCA group; $^{\blacktriangle\blacktriangle}P$<0.01, compared with model+RSV group.

2.7 奥贝胆酸与白藜芦醇联用对NAFLD模型小鼠肝组织Sirt1,NF-κB和p-NF-κB蛋白表达水平的影响

由图3可知,与正常对照组相比,模型组肝组织

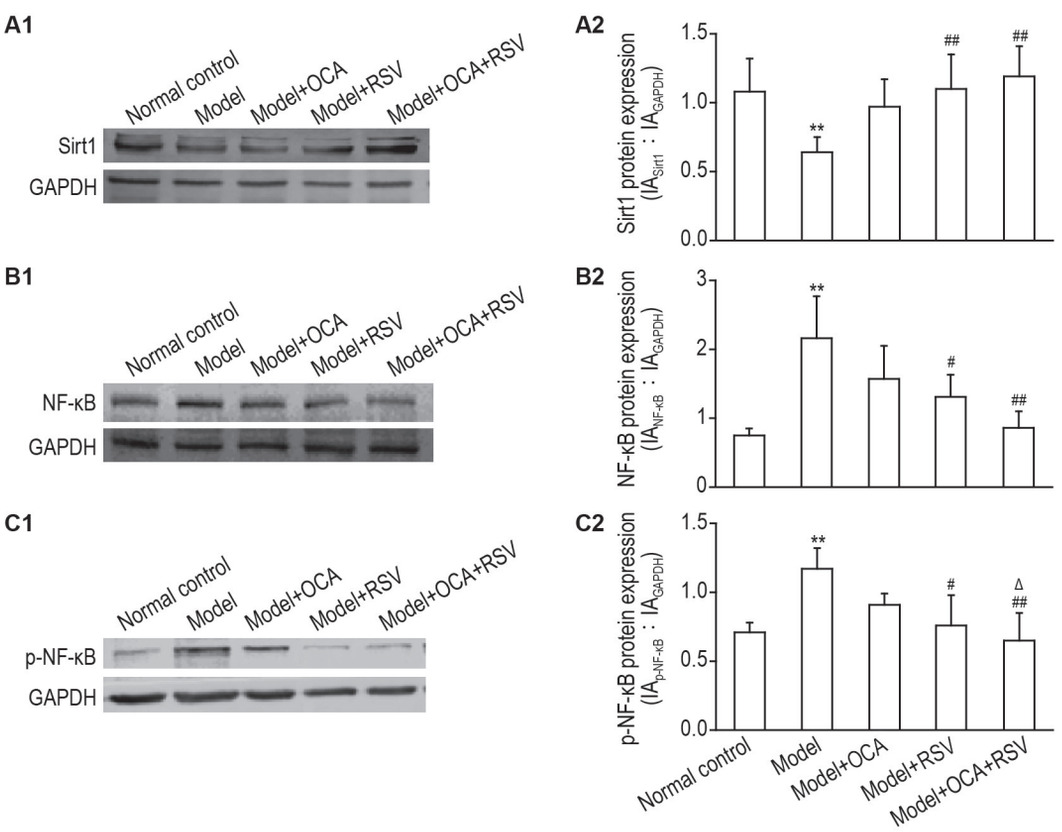

Fig.3 Effect of OCA combined with RSV on protein expression levels of sirtuin 1 (Sirt1) (A), NF-κB (B) and phosphorylated NF-κB (p-NF-κB) (C) in liver tissue of mice with NAFLD by Western blotting. See Tab.1 for the mouse treatment. A2, B2 and C2 were the semiquantitative results of A1, B1 and C1, respectively. $\bar{x}\pm s$, n=8. **P<0.01, compared with normal control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, compared with model group; $^{\triangle}P$<0.05, compared with model+OCA group.

Sirt1蛋白表达水平明显降低，NF-κB和p-NF-κB表达水平明显增加（*P*<0.01）。与模型组相比，模型+OCA组Sirt1，NF-κB和p-NF-κB蛋白表达水平无明显变化；模型+RSV组Sirt1蛋白表达水平明显增加（*P*<0.01），NF-κB和p-NF-κB蛋白表达水平明显降低（*P*<0.05）；模型+OCA+RSV组Sirt1蛋白表达水平明显增加（*P*<0.01），NF-κB和p-NF-κB蛋白表达水平明显降低（*P*<0.01），且p-NF-κB蛋白表达水平较模型+OCA组明显降低（*P*<0.05）。

3 讨论

本研究结果表明，OCA与RSV联用可降低NAFLD模型小鼠血清MDA水平，同时Sirt1蛋白表达水平升高，NF-κB表达水平降低，IL-6，IL-1β和TNF-α分泌降低。有研究表明，细胞内抗氧化剂的消耗和炎症反应在NAFLD形成中具有重要作用，是对肝“二次打击”的主要形式，随后肝细胞死亡和细胞凋亡，促使NAFLD向NASH进展[14]，过氧化产物MDA含量可体现细胞受氧自由基损伤程度[15]。NF-κB是至关重要的核转录因子，促进炎症因子IL-6，IL-1β和TNF-α分泌[16]。高脂饮食可激活NF-κB，升高肝组织IL-6，IL-1β和TNF-α水平[17]。Sirt1是一种烟酰胺腺嘌呤二核苷酸（NAD）依赖性酶[18]，Sirt1可使NF-κB 亚基去乙酰化，并抑制其转录活性[19]，减少炎症因子分泌，抑制炎症对NAFLD的肝损伤，而阻止从 NAFLD进展为NASH。本研究结果表明，OCA与RSV联用清除体内MDA能力增加，减轻肝脂质过氧化，提高抗氧化能力，同时增加Sirt1表达并抑制NF-κB激活，提示OCA与RSV联用可能通过Sirt1/NF-κB途径减少IL-6，IL-1β和TNF-α等炎症因子分泌，降低 NAFLD氧化应激对肝“二次打击”和炎症因子对肝的损伤。

本研究结果表明，OCA与RSV联用在改善NAFLD组织和血清指标的同时，可更显著降低TC和LDL水平，升高HDL水平，与前期相关研究结果[20]一致。有研究表明，OCA在降低NAFLD患者肝脂肪水平时，血清LDL和TC升高，HDL降低，从而使心血管疾病风险增加[8]。TC增加会导致动脉硬化，诱发心血管疾病，可作为准确预测心血管风险的指标[20-21]，同时HDL降低和LDL升高也是心血管疾病发病的重要因素[22]。本研究结果表明，OCA与RSV联用还改善了OCA在脂蛋白和血脂调节能力的不足，从而降低了OCA单用时TC和LDL升高、HDL降低导致的心脑血管等风险。

综上所述，OCA与RSV联用可提高抗氧化应激能力，并通过调节Sirt1和NF-κB蛋白表达及NF-κB活化减少炎症因子分泌，从而减轻NAFLD模型小鼠肝损伤，阻止NAFLD进一步发展；还可调节血脂和脂蛋白，降低OCA单用带来的心血管疾病风险。本研究结果为OCA与RSV联用治疗NAFLD提供了实验依据。

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Therapeutic effect of obeticholic acid combined with resveratrol against non-alcoholic fatty liver disease in mice

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Abstract: OBJECTIVE To explore the therapeutic effect and mechanism of obeticholic acid (OCA) combined with resveratrol (RSV) in model mice with non-alcoholic fatty liver disease (NAFLD). **METHODS** Forty-two male C57BL/6N mice, except the normal control group, were injected with 1% CCl_4 by ip 5 mL·kg^{-1} once a week and fed with high-fat diet for four weeks to induce an NAFLD model. The model

mice were divided into the model group, model+OCA group, model+RSV group and model+OCA+RSV group, and ig administered with or without OCA 30 mg·kg^{-1} or (and) RSV 30 mg·kg^{-1}, once a day, for 28 d. The mice were sacrificed, blood was collected, and the serum was collected by centrifugation. The liver tissues were collected to calculate the liver index. The pathological changes of the liver tissue of the mice were observed by HE staining. The serum levels of glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), superoxide dismutase (SOD), malondialdehyde (MDA), interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) were detected by automatic biochemical analyzer and detection kits. The protein expression levels of sirtuin 1 (Sirt1), NF-κB and p-NF-κB in liver tissue were detected by Western blotting. **RESULTS** Compared with the normal control group, the liver indexes of model group were increased significantly (P<0.01). Compared with the model group, the liver indexes of drug treated groups were decreased significantly (P<0.01). Compared with the model+RSV group, the liver indexes of the model+OCA+RSV group were decreased significantly (P<0.05). The liver tissue sections of the mice in the model group had a large number of fat vacuoles and inflammatory lesions, and a large number of red fat deposits were found via oil red O staining. The levels of serum GPT, GOT, TC, LDL, MDA, IL-1β and IL-6, the level of TNF-α and the expression and phosphorylation of NF-κB in liver tissue were significantly increased (P<0.01), while the level of serum HDL and the expression of Sirt1 in liver tissue were significantly decreased (P<0.05). Compared with the model group, the liver cells and tissues were normal and there were a few steatotic vacuoles and red fat deposition decreased in the model+OCA group. Serum GOT, TC, MDA, IL-6 and TNF-α levels were significantly decreased (P<0.05), GPT, HDL and LDL levels did not change significantly. In the model+RSV group, the fat droplet vacuoles were reduced significantly, the red lipid droplets were not reduced significantly, serum TC, LDL, MDA, IL-1β, IL-6 and TNF-α levels and the phosphorylation level of NF-κB in liver tissue were significantly decreased (P<0.05), the expression level of Sirt1 in liver tissue was significantly increased (P<0.05), but the serum levels of GPT, GOT and HDL were not significantly changed. In the model+OCA+RSV group, the lipid droplet vacuoles were significantly reduced, so was lipid deposition, serum levels of GPT, GOT, TC, LDL, MDA, IL-1β, IL-6 and TNF-α, and the expression and phosphorylation of liver tissue NF-κB (P<0.01), but serum HDL levels and tissue Sirt1 expression level were significantly increased (P<0.01). Compared with the model+OCA group, the levels of GPT, TC, LDL, MDA, IL-1β and IL-6 in serum, and phosphorylation of NF-κB in liver tissue were significantly decreased (P<0.05), but the serum level of HDL was significantly increased (P<0.01) in the model+OCA+RSV group. Compared with the model+RSV group, the lipid deposition in liver tissue and serum levels of GPT, GOT and MDA were significantly decreased (P<0.05), but the serum HDL level was significantly increased (P<0.05) in the model+OCA+RSV group. **CONCLUSION** The combined application of OCA and RSV can increase the anti-oxidative stress capacity and reduce the inflammatory response by regulating the expressions of Sirt1 and NF-κB so that the development of NAFLD is inhibited.

Key words: obeticholic acid; non-alcoholic fatty liver; resveratrol; oxidative stress

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