

白芍总苷对糖皮质激素致肝损伤的保护作用及初步机制

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摘要 **目的** 探讨白芍总苷(TGP)对地塞米松(DEX)致大鼠肝损伤的保护作用。**方法** 30只SD大鼠随机分为正常(N)、DEX、DEX+TGP(50 mg/kg)、DEX+TGP(100 mg/kg)、DEX+TGP(200 mg/kg)组,每组6只。采用腹腔注射DEX(17.5 mg/kg)的方法构建大鼠肝损伤模型,检测大鼠血清中丙氨酸氨基转移酶(ALT)、天门冬氨酸氨基转移酶(AST)、碱性磷酸酶(ALP)水平,计算肝体比;HE染色观察肝脏病理学变化,测定肝组织丙二醛(MDA)、超氧化物歧化酶(SOD)、谷胱甘肽(GSH)含量,Western blot检测肝组织NADPH氧化酶4(NOX4)、内质网应激相关蛋白:葡萄糖调节蛋白94(GRP94)、GRP78、磷酸化真核起始因子2 α (p-eIF2 α)、CCAAT/增强子结合蛋白同源蛋白(CHOP)表达水平。**结果** 与正常组相比,DEX组中ALT、AST、ALP、肝体比水平升高($t=14.96, 9.87, 13.48, 11.45, P<0.01$),肝组织病理学表现明显肝细胞肿胀变性、坏死($t=15.49, P<0.01$);与DEX组相比,TGP(50 mg/kg)治疗组肝功能生化指标、肝组织病理无明显变化,TGP(100、200 mg/kg)治疗组ALT、AST、ALP、肝体比($t=3.30, 4.13, 7.45, 2.97, P<0.05; t=8.92, 6.45, 8.65, 7.47, P<0.01$)水平下降,肝组织病理评分降低($t=4.33, P<0.05; t=5.63, P<0.01$)。氧化应激指标显示,DEX组MDA、NOX4水平升高($t=7.06, 4.23, P<0.01$),SOD、GSH水平降低($t=7.78, 7.92, P<0.01$);TGP(50 mg/kg)治疗组无明显变化,TGP(100、200 mg/kg)治疗组MDA、NOX4($t=3.35, 4.30, P<0.05; t=5.44, 7.44, P<0.01$)水平降低,SOD($t_{200\text{ mg/kg}}=4.04, P<0.05$)、GSH($t=4.70, P<0.05; t=5.50, P<0.01$)水平上升。与正常组相比,DEX组内质网应激蛋白GRP94、GRP78、p-eIF2 α 、CHOP($t=3.31, 6.53, 5.18, 3.09, P<0.05, 0.01, 0.01, 0.05$)表达水平升高;与DEX组相比,TGP(100、200 mg/kg)治疗组GRP94、GRP78、p-eIF2 α 、CHOP($t=3.14, 4.95, 3.13, 4.25, P<0.05; t=4.03, 7.48, 4.68, 5.10, P<0.01$)表达水平下降,TGP(50 mg/kg)治疗组无明显变化。**结论** TGP对DEX肝损伤有一定的保护作用,其机制与抑制DEX诱导大鼠肝脏氧化应激和内质网应激有关。

关键词 白芍总苷;糖皮质激素;地塞米松;肝损伤;氧化应激;内质网应激

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糖皮质激素(glucocorticoids, GCs)具有抗炎、抗过敏等作用,临床上广泛用于各种过敏和自身免疫性疾病防治^[1]。地塞米松(dexamethasone, Dex)是临床应用最广泛的GCs之一,但大剂量或长期用药会导致不可逆的肝损伤^[2],临床表现为黄疸、恶心等消化道症状,严重时出现肝肿大或腹水;病理特征为肝细胞水肿、局灶性坏死及脂肪变性,其病理过程具有剂量和时间依赖性。研究^[3]表明,药物性肝损伤通过扰乱内质网稳态,激活内质网应激(endoplas-

mic reticulum stress, ERS)及凋亡途径,上调相关标志蛋白与促凋亡信号分子,驱动肝细胞程序性死亡。白芍总苷(total glucosides of paeony, TGP)是从芍药根部提取的有效成分,具有调节免疫及抗氧化特性,副作用小,临床作为免疫调节剂广泛用于自身免疫疾病及肾脏疾病辅助治疗^[4]。该研究通过大剂量DEX给药建立大鼠肝损伤模型,再给予TGP干预,考察TGP对DEX致肝损伤的作用,初步揭示TGP和DEX之间的“减毒作用”,为防治DEX致肝损伤提供依据。

1 材料与方法

1.1 药物与试剂 TGP胶囊购自宁波立华制药有限公司(批号:H20055058);地塞米松磷酸钠注射液购自山东辰欣药业股份有限公司(批号:H37021969);丙氨酸氨基转移酶(alanine aminotransferase, ALT)(货号:C009-2-1),天门冬氨酸氨基转移酶(aspartate aminotransferase, AST)(货号:

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C010-2-1),碱性磷酸酶(alkaline phosphatase, ALP)(货号:A059-1-1),丙二醛(malondialdehyde, MDA)(货号:A003-1-1),总超氧化物歧化酶(superoxide dismutase, SOD)(货号:A001-3-1),还原型谷胱甘肽(reduced glutathione, GSH)(货号:A006-2-1)检测试剂盒均购自南京建成生物工程研究所;NADPH 氧化酶4(reduced nicotinamide adenine dinucleotide phosphate oxidase 4, NOX4)抗体(货号:NB11058849SS)购自美国 Novus Biologicals 公司;葡萄糖调节蛋白94(glucose regulatory protein 94, GRP94)(货号:AF5287)、GRP78(货号:AF5366)、p-eIF2 α (货号:AF3087)、CCAAT/增强子结合蛋白同源蛋白(CCAAT/enhancer binding protein homologous protein, CHOP)(货号:AF5280)、 β -actin(货号:AF7018)购自美国 Affinity Biosciences 公司;HRP 标记的山羊抗兔 IgG 二抗(货号:C31460100)购自美国 Proteintech 公司。

1.2 实验仪器 ELx808 酶标仪(美国 Bio Tek 公司);组织原位细胞扫描分析系统(山东 3DHISTECH 公司);3-30K 低温高速离心机(美国 Sigma 公司);自动化学发光图像分析系统(Tanon5200,上海 Tanon 公司)。

1.3 动物分组与模型制备 清洁级雄性 SD 大鼠 30 只,6~8 周龄,体质量(200 ± 20)g,由斯贝福(江苏)生物技术有限公司提供[合格证号:SCXK(苏)2022-006]。该动物实验获得安徽医科大学临床药理研究所动物实验伦理委员会批准(批号:PZ-2023-028)。实验动物随机分为正常、DEX[17.5 mg/(kg·d)]、DEX + TGP[50 mg/(kg·d)]、DEX + TGP[100 mg/(kg·d)]、DEX + TGP[200 mg/(kg·d)]组,每组 6 只,其中治疗药物 TGP 在 DEX 注射前 7 d 开始灌胃给药,正常组和 DEX 组大鼠给予等体积的溶媒。第 8 天开始,DEX 组和 DEX + TGP 组腹腔注射 DEX [17.5 mg/(kg·d)],正常组腹腔注射相同体积生理盐水,连续注射 3 d,诱导肝损伤模型,TGP 继续灌胃给药,实验共持续 10 d。

1.4 检测指标

1.4.1 肝功能生化指标 第三次注射 DEX 24 h 后,大鼠称重后收集外周血,分离血清。采用相关试剂盒检测大鼠血清 ALT、AST 和 ALP 水平。血清中 ALT/AST 活力(U/L)=[绝对吸光度(optical density, OD)值(测定 OD - 对照 OD)根据标准曲线计算得卡门氏单位] $\times 0.482 \times$ 样本测试前稀释倍数;血清中 ALP 活力(金氏单位/100 mL) = 测定 OD/标准

OD $\times$ 标准管含酶量 0.005 mg $\times$ 100 mL/血清取样量 50 μ L。

1.4.2 肝脏组织病理学 处死大鼠后,取肝脏称重,取部分肝脏组织,置于 4 % 多聚甲醛溶液中固定,进行 HE 染色。肝脏组织病理学评分参照以下标准进行:正常肝组织细胞未见病变(0 分);少部分肝细胞坏死,或伴有肝细胞轻微肿胀(1 分);局灶性肝细胞肿胀变性、坏死(2 分);超过 50 % 的肝细胞肿胀变性、坏死(3 分);超过 75 % 的肝细胞肿胀变性、坏死(4 分)^[5]。

1.4.3 肝脏氧化应激水平检测 准确称取肝脏组织,按照质量(g):体积(mL) = 1:9 的比例加入生理盐水,剪碎组织,使用自动匀浆机制备组织匀浆;得到的组织匀浆离心 10 min,取匀浆上清液用于后续测定,使用蛋白测量仪测定蛋白浓度,用于计算。按照 MDA、SOD 和 GSH 试剂盒说明书分别在 532、450 和 405 nm 处对 MDA、SOD 和 GSH 含量进行测定。组织中 MDA 的含量(nmol/mgprot) = (测定 OD - 对照 OD)/(标准 OD - 空白 OD) $\times$ 标准品浓度(10 nmol/mL)/待测样本蛋白浓度(mgprot/mL);SOD 的抑制率:SOD 抑制率(%) = (对照 OD - 测定 OD - 测定空白 OD)/(对照 OD - 对照空白 OD) $\times$ 100%;SOD 活力:SOD 活力(U/mgprot) = SOD 抑制率/50 % $\times$ 反应体系稀释倍数 $\div$ 待测样本蛋白浓度(mgprot/mL);组织 GSH 含量(μ mol/gprot) = (测定 OD - 空白 OD)/(标准 OD - 空白 OD) $\times$ 标准管浓度 $\times$ 样本前处理稀释倍数 $\div$ 待测匀浆蛋白浓度(gprot/L)。

1.4.4 Western blot 实验 取部分大鼠肝脏组织,使用 RIPA 裂解液裂解肝组织后提取总蛋白,BCA 法进行蛋白定量。取适量蛋白样本,10 % 十二烷基硫酸钠/聚丙烯酰胺凝胶电泳将其分离,并转移到 PVDF 膜上。用 5 % 脱脂牛奶封闭 2 h 后,一抗 4 $^{\circ}$ C 孵育过夜:NOX4(1:500)、GRP94(1:1 000)、GRP78(1:1 000)、p-eIF2 α (1:1 000)、CHOP(1:1 000);二抗(1:20 000)室温孵育 2 h,通过发光成像分析仪显影,最后使用 ImageJ 软件对蛋白条带进行分析。

1.5 统计学处理 采用 GraphPad Prism 8.0 进行数据分析及绘图,所有数据均用 $\bar{x} \pm s$ 表示;采用单因素方差分析(One-way ANOVA)进行多组间均数比较,采用最小显著性差异(least significant difference, LSD)检验进行两两比较, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 TGP 对 DEX 致大鼠肝损伤肝功能生化指标的影响 采用相关试剂盒检测大鼠血清样本中 ALT、AST、ALP ($F = 75.71$ 、 31.96 、 60.59 , 均 $P < 0.05$) 水平,如图 1A、B、C 所示,与正常组相比,DEX 组大鼠血清 ALT、AST、ALP 水平显著升高 ($t = 14.96$ 、 9.87 、 13.48 , $P < 0.01$);与 DEX 组相比,TGP (100、200 mg/kg) 治疗组大鼠血清 ALT、AST、ALP ($t = 3.30$ 、 4.13 、 7.45 , 均 $P < 0.05$; $t = 8.92$ 、 6.45 、 8.65 , 均 $P < 0.01$) 水平明显下降,差异有统计学意义。通过对大鼠肝体比的计算及统计,如图 1D 所示,DEX 组大鼠肝体比较正常组明显升高 ($t = 11.45$, $P < 0.01$),与 DEX 组相比,TGP (100、200 mg/kg) 治疗组大鼠肝体比下降 ($t = 2.97$, $P < 0.05$; $t = 7.47$, $P < 0.01$),差异有统计学意义。实验结果提示,TGP 对 DEX 致大鼠肝损伤具有一定的保护作用。

2.2 TGP 对 DEX 致大鼠肝损伤组织病理学的影响 如图 2 所示,HE 染色结果显示 ($F = 74.53$, $P < 0.01$),正常组大鼠肝脏组织正常,肝细胞排列整齐、未见细胞水肿变性、坏死。与正常组相比,DEX 组大鼠出现肝损伤,肝细胞广泛性水肿变性、坏死 ($t = 15.49$, $P < 0.01$)。TGP (100、200 mg/kg) 治疗组大鼠肝组织细胞坏死减少、水肿得到改善,组织病理学评分低于 DEX 组 ($t = 4.33$, $P < 0.05$; $t = 5.63$, $P < 0.01$),差异有统计学意义。

2.3 TGP 对 DEX 致大鼠肝损伤组织氧化应激水平的影响 大鼠肝脏组织中氧化应激指标 MDA、SOD、GSH 水平 ($F = 22.56$ 、 23.94 、 22.31 , 均 $P < 0.05$),见图 3A、B、C,与正常组相比,DEX 组 MDA 水平明显升高 ($t = 7.06$, $P < 0.01$),SOD、GSH 活力下降 ($t = 7.78$ 、 7.92 , $P < 0.01$),差异有统计学意义;与 DEX 组相比,TGP (100、200 mg/kg) 治疗组 MDA ($t = 3.35$, $P < 0.05$; $t = 5.44$, $P < 0.01$) 水平下降,SOD ($t_{200 \text{ mg/kg}} = 4.04$, $P < 0.01$)、GSH ($t = 4.70$,

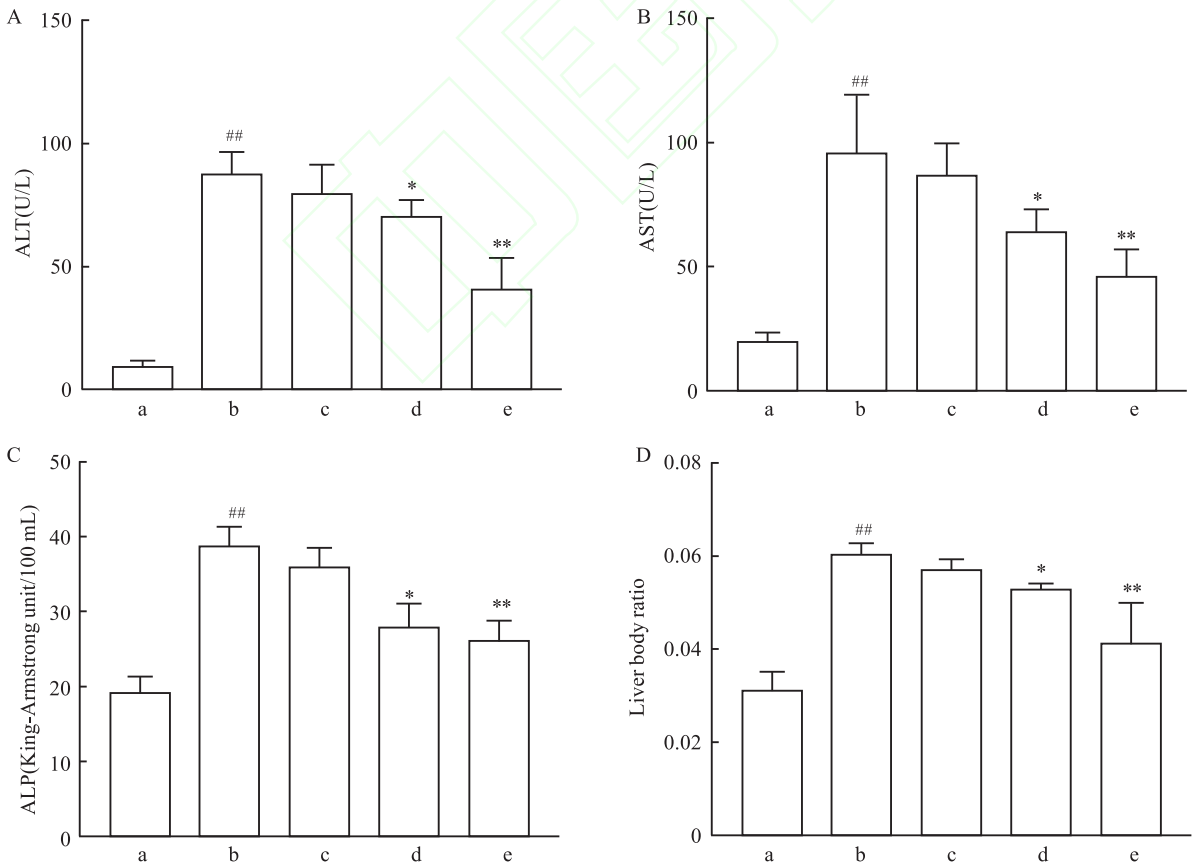


图 1 TGP 对 DEX 致大鼠肝损伤生化指标的影响

Fig. 1 The effects of TGP on biochemical indexes of DEX-induced liver injury in rats

A: Rat serum ALT; B: Rat serum AST; C: Rat serum ALP; D: Rat liver body ratio; a: Normal group; b: DEX group; c: DEX + TGP (50 mg/kg); d: DEX + TGP (100 mg/kg); e: DEX + TGP (200 mg/kg); ^{##} $P < 0.01$ vs Normal group; ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs DEX group.

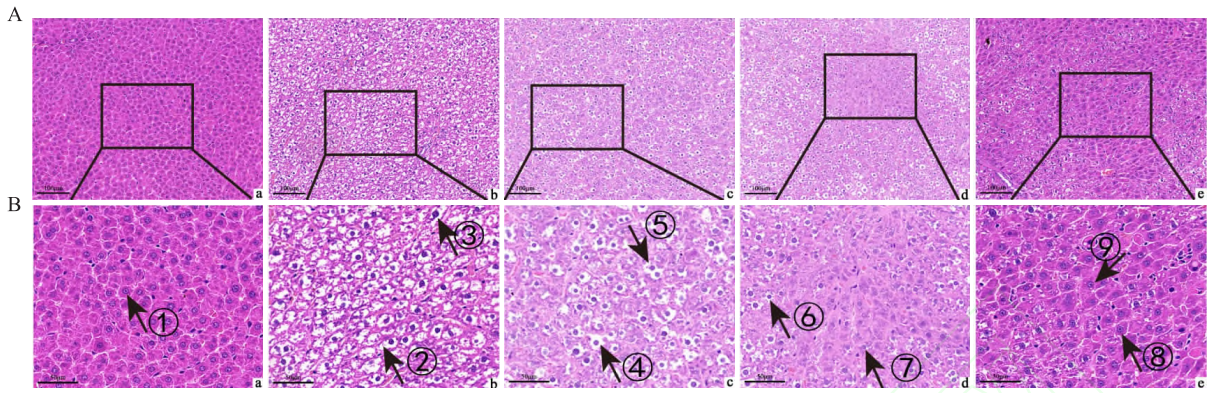


图2 TGP对DEX致大鼠肝损伤组织病理学的影响

Fig.2 The effects of TGP on histopathology of DEX-induced liver injury in rats

A: HE staining $\times 400$; B: HE staining $\times 1\,000$; ①: normal hepatocytes; ②~⑤: hepatocyte edema, degeneration and necrosis; ⑥: hepatocyte edema is relieved; ⑦~⑨: Normal hepatocytes); C: Histopathology score; a: Normal group; b: DEX Group; c: DEX + TGP (50 mg/kg) group; d: DEX + TGP (100 mg/kg) group; e: DEX + TGP (200 mg/kg) group; $^{##}P < 0.01$ vs Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs DEX group.

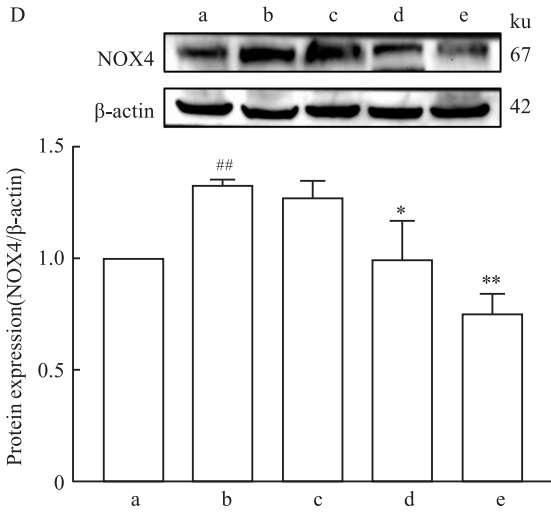
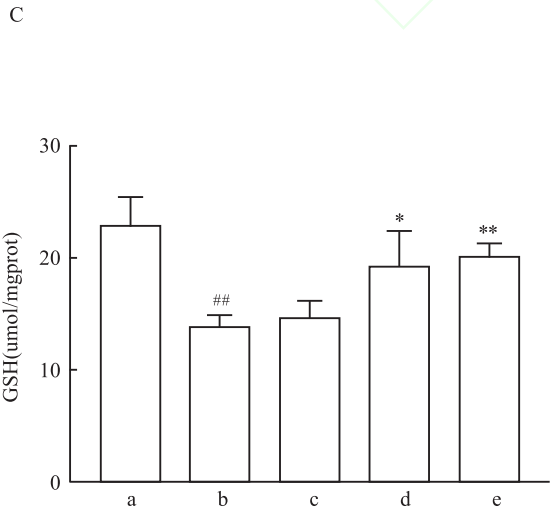
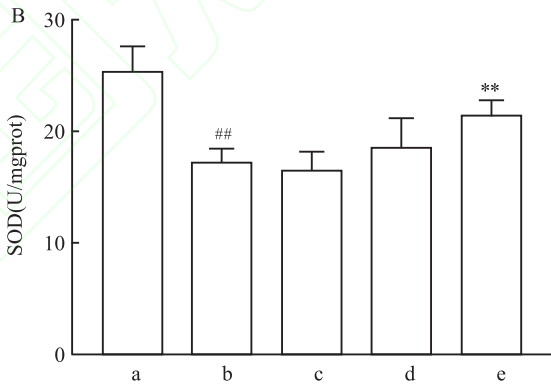
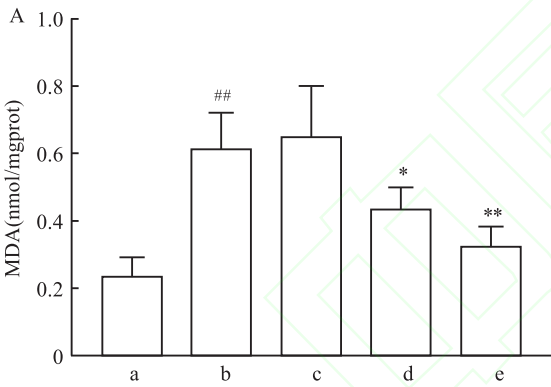
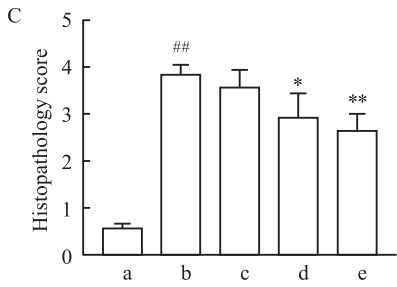


图3 TGP对DEX致大鼠肝损伤组织氧化应激水平的影响

Fig.3 The effects of TGP on oxidative stress levels in rat liver injury tissues induced by DEX

A: Rat liver tissue MDA; B: Rat liver tissue SOD; C: Rat liver tissue GSH; D: Western blot method was used to detect the expression of NOX4 protein in liver tissue of rats in each group; a: Normal group; b: DEX Group; c: DEX + TGP (50 mg/kg) group; d: DEX + TGP (100 mg/kg) group; e: DEX + TGP (200 mg/kg) group; $^{##}P < 0.01$ vs Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs DEX group.

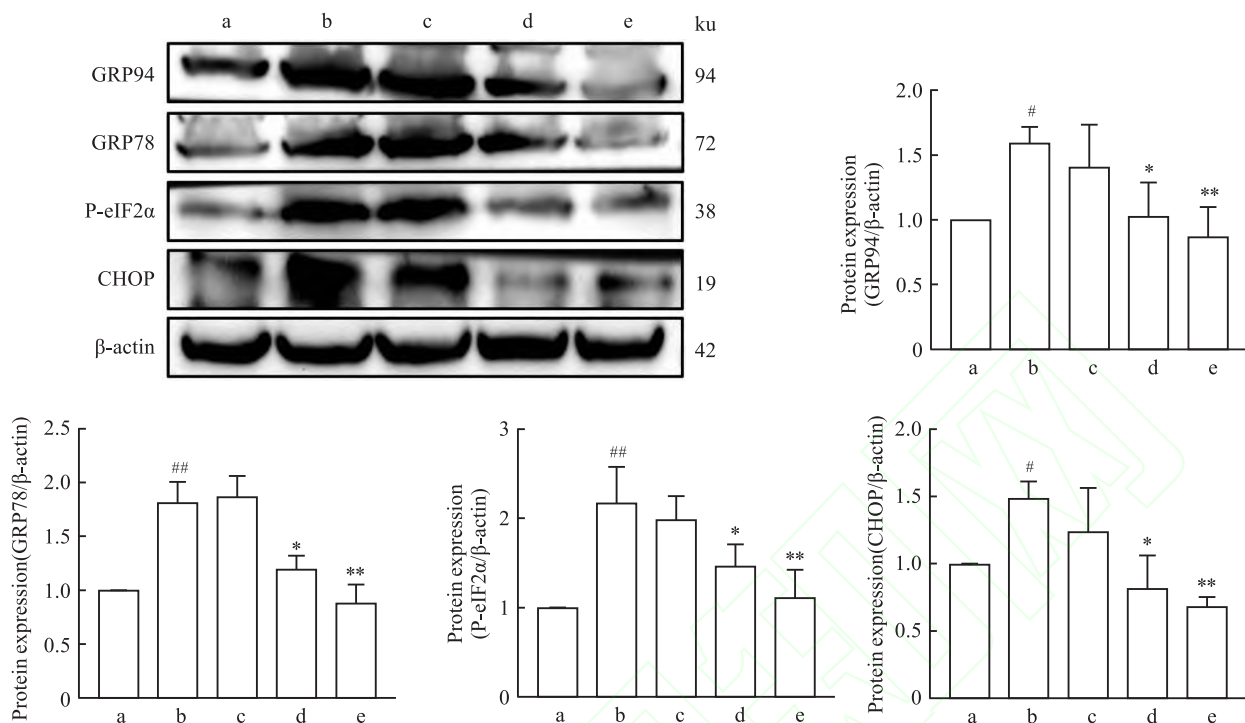


图4 TGP对DEX致大鼠肝损伤组织内质网应激蛋白表达的影响

Fig. 4 The effects of TGP on the expression of endoplasmic reticulum stress protein in rat liver injury induced by DEX

[#] $P < 0.05$, ^{##} $P < 0.01$ vs Normal group; ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs DEX group.

$P < 0.05$; $t = 5.50$, $P < 0.01$) 水平上升, 差异有统计学意义。Western blot 法检测各组大鼠肝组织 NOX4 表达水平 ($F = 18.24$, $P < 0.01$), 见图 3D, 与正常组相比, DEX 组 NOX4 水平升高 ($t = 4.23$, $P < 0.01$), 差异有统计学意义; 与 DEX 组相比, TGP (100、200 mg/kg) 治疗组 NOX4 水平下降 ($t = 4.30$, $P < 0.05$; $t = 7.44$, $P < 0.01$), 差异有统计学意义。

2.4 TGP 对 DEX 致大鼠肝损伤组织内质网应激蛋白表达的影响 Western blot 法检测各组大鼠肝组织内质网应激相关蛋白 GRP94、GRP78、p-eIF2α、CHOP 表达 ($F = 5.78$ 、27.23、10.50、8.41, 均 $P < 0.05$), 见图 4, 与正常组比较, DEX 组大鼠肝组织 GRP94、GRP78、p-eIF2α、CHOP ($t = 3.31$ 、6.53、5.18、3.09, $P < 0.05$ 、0.01、0.01、0.05) 表达上升, 差异有统计学意义; 与 DEX 组相比, TGP (100、200 mg/kg) 治疗组 GRP94 ($t = 3.14$ 、4.95、3.13、4.25, $P < 0.05$; $t = 4.03$ 、7.48、4.68、5.10, $P < 0.01$)、GRP78、p-eIF2α、CHOP 表达下降, 差异有统计学意义。

3 讨论

DEX 具有抗炎、免疫抑制等药理作用^[6], 很多研究^[7-8]将 DEX 作为保肝药物的阳性对照药, 但大

剂量使用可致中毒性肝损伤, 甚至诱发脂肪肝。根据《糖皮质激素临床应用指导原则》^[9], 临床使用 GCs 实施短期冲击剂量治疗时, 疗程多小于 5 d, 甲泼尼龙临床冲击剂量为 7.5 ~ 30 mg/(kg · d)。根据甲泼尼龙和 DEX 剂量换算比 (4 : 0.75) 及人 - 大鼠体表面积剂量换算比 (1 : 6.3), 确定大鼠 DEX 造模剂量为 8.86 ~ 35.44 mg/(kg · d)。前期预实验显示, 选用冲击剂量中间值 17.5 mg/(kg · d) 腹腔注射 3 d, 大鼠死亡率为 0, 造模成功率为 100%, 符合临床应用情况。DEX 作用后, ALT、AST、ALP、肝体比水平均高于正常组, HE 结果显示多数肝细胞水肿变性、坏死。

TGP 是从天然中药白芍中提取的有效成分, 具有天然药物的特点和优势, 与化学合成药物相比, 长期应用 TGP 具有较低的毒副作用和更好的耐受性^[10]。已有研究^[11]发现 TGP 对化学性肝损伤 (如酒精性肝损伤、四氯化碳肝损伤) 或免疫性肝损伤的保护作用, 在此基础上, 该研究首次探讨 TGP 对 GCs 特异性肝损伤的干预效应。本实验中, TGP (100、200 mg/kg) 能显著降低 ALT、AST、ALP 和肝体比水平, 说明 TGP 具有良好的肝保护作用。大量研究^[1]表明, 高剂量 DEX 会增加脂质过氧化, 产生活性氧, 抑制抗氧化酶, 从而导致氧化损伤, 产生肝

毒性;DEX 发挥抗感染作用的同时,也可能抑制肝脏的免疫反应,这可能会影响肝脏对损伤的修复能力^[7]。免疫功能的异常改变和氧自由基的大量产生是引起肝组织损伤的重要原因,而 TGP 可以通过免疫调节和抗氧化作用显著改善肝组织急性损伤^[4]。MDA、SOD、GSH 和 NOX4 是氧化应激机制中抗氧化系统的重要检测指标,能够反映出机体抗氧化作用的潜在能力及损伤程度^[5]。本实验中 DEX 组 MDA、NOX4 水平升高,SOD、GSH 水平降低,TGP (100、200 mg/kg) 治疗组 MDA、NOX4 水平降低,SOD 和 GSH 水平升高,说明注射大剂量的 DEX 时,大鼠肝脏发生氧化应激反应,造成氧化损伤,TGP 能够明显抑制 DEX 诱导的氧化应激反应。上述结果提示,TGP 能够减轻氧化应激对肝脏造成的损伤。

ERS 是以内质网腔内错误折叠和未折叠的蛋白质异常积累为特征的细胞过程^[12]。在急性肝损伤发生时,肝细胞蛋白质合成和代谢紊乱导致错误折叠的蛋白质在内质网中积累,破坏内质网稳态,进而激活未折叠蛋白质反应(unfolded protein response, UPR)及其关键信号通路分子^[13]。研究^[14]表明,内质网应激与多种原因引起的肝脏损伤有关,肝脏受损时,内质网应激中的 PERK-eIF2 α -CHOP 途径是重要的促细胞凋亡分支。该实验中,DEX 显著上调大鼠肝组织内质网应激相关蛋白 GRP94、GRP78、p-eIF2 α 、CHOP 表达,而 TGP (100、200 mg/kg) 可有效逆转上述蛋白异常表达。既往研究^[11,15]中,TGP 主要通过激活 Nrf2 以及抑制 NF- κ B 调控氧化应激和炎症免疫相关信号通路,如在化学性肝损伤模型中,TGP 通过激活 Nrf2 通路减轻氧化应激损伤进而维持肝细胞功能稳态、通过抑制 NF- κ B 减少炎症因子释放发挥抗炎作用,从而在化学性肝损伤中发挥肝保护作用。该研究首次发现 TGP 通过抑制 ERS 相关凋亡通路减轻细胞损伤和死亡,改善 ERS 相关蛋白的表达。上述结果提示,高剂量 DEX 会引起大鼠肝脏发生 ERS,TGP 能够通过调控 ERS 相关蛋白发挥肝保护作用。

尽管 GCs 因其强效抗炎与免疫抑制作用在临床治疗中不可替代,但其肝毒性限制了长期或大剂量应用。因此,该研究进一步提出 TGP 作为 GCs 在临床治疗的协同保护剂,而非传统保肝药物的独立治疗模式,在保留 GCs 疗效的同时减轻其肝毒性,凸显 TGP 在联合用药中的临床转化潜力。此外,临床上常用的保肝药物种类繁多,TGP 与其他保肝药物或治疗方法具有协同作用,例如,与抗氧化剂联合

使用时,TGP 的抗氧化作用可能与其他抗氧化剂相互补充,增强抗氧化效果^[4]。因此,TGP 在联合治疗中具有一定的应用潜力,可能为 DEX 致肝损伤的防治提供新的思路和方法。

综上所述,TGP 对 DEX 致肝损伤具有保护作用,可用于 DEX 致肝损伤的防治,其作用机制可能与抑制肝组织氧化应激反应和降低内质网应激水平有关。其具体分子机制还有待进一步研究。

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Protective effect of total glucosides of paeony on glucocorticoid-induced liver injury and preliminary mechanisms

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Abstract Objective To investigate the protective effect of total glucosides of paeony (TGP) on dexamethasone (DEX)-induced liver injury in rats. **Methods** Thirty SD rats were randomly divided into normal (N), DEX, DEX + TGP (50 mg/kg), DEX + TGP (100 mg/kg), and DEX + TGP (200 mg/kg) groups, with 6 rats in each group. A rat model of liver injury was established by intraperitoneal injection of DEX (17.5 mg/kg). Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured, and the liver-to-body weight ratio was calculated. HE staining was performed to observe histopathological changes in the liver. The contents of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) in liver tissues were determined. Western blot analysis was conducted to detect the expression levels of NADPH oxidase 4 (NOX4) and endoplasmic reticulum stress-related proteins: glucose regulatory protein 94 (GRP94), GRP78, phosphorylated eukaryotic initiation factor 2 α (p-eIF2 α), CCAAT/enhancer binding protein homologous protein (CHOP) expression level in liver tissues. **Results** Compared with the normal group, the DEX group exhibited significantly elevated serum levels of ALT, AST, ALP and liver-to-body weight ratio ($t = 14.96, 9.87, 13.48, 11.45, P < 0.01$), along with marked histopathological damage characterized by hepatocyte swelling, degeneration, and necrosis ($t = 15.49, P < 0.01$). Compared with the DEX group, there was no significant change in liver function biochemical indexes and liver histopathology in the TGP (50 mg/kg) treatment group. TGP treatment at 100 mg/kg and 200 mg/kg significantly attenuated these effects: both doses reduced ALT, AST, ALP and liver-to-body weight ratio ($t = 3.30, 4.13, 7.45, 2.97, P < 0.05; t = 8.92, 6.45, 8.65, 7.47, P < 0.01$), while improving histopathological scores ($t = 4.33, P < 0.05; t = 5.63, P < 0.01$). Oxidative stress analysis revealed that DEX administration significantly increased hepatic MDA levels ($t = 7.06, P < 0.01$) and NOX4 expression ($t = 4.23, P < 0.01$), whereas SOD activity ($t = 7.78, P < 0.01$) and GSH content ($t = 7.92, P < 0.01$) were markedly suppressed. There was no significant change in the TGP (50 mg/kg) treatment group, TGP

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death induced by ES + Cu²⁺ was detected by flow cytometry and the changes of ES + Cu²⁺ after pretreatment with copper chelating agent ATTM. The expression of Cuproptosis related proteins ATPase copper transporting beta (ATP7B), ferredoxin 1 (FDX1), dihydrolipoamide S-acetyltransferase (DLAT) and superoxide dismutase 1 (SOD1) were detected by Western blot. The effect of ES + Cu²⁺ on cell proliferation and the reverse effect after ATTM pretreatment was detected by cell scratch assay. **Results** The toxicity of ES + Cu²⁺ to human hepatocellular carcinoma cell lines PLC/PRF/5 and Huh-7 was significantly dose-dependent ($P < 0.05$). Compared with the control group, the combined application of ES and Cu²⁺ had a more significant inhibitory effect on hepatocellular carcinoma cells than ES or Cu²⁺ alone ($P < 0.05$), and copper chelating agent ATTM could reverse the inhibitory effect of ES + Cu²⁺ on hepatocellular carcinoma cells ($P < 0.05$). Flow cytometry results showed that compared with the control group, the proportion of cell death in PLC/PRF/5 and Huh-7 cells treated with ES + Cu²⁺ increased, while the proportion of cell death decreased after ATTM intervention ($P < 0.05$). The results of cell scratch test showed that the migration ability of PLC/PRF/5 and Huh-7 cells was decreased after ES + Cu²⁺ treatment, however, the addition of ATTM reversed the inhibitory effect of ES + Cu²⁺ on cell migration ($P < 0.05$). Compared with the control group, the expression levels of copper death related proteins ATP7B, FDX1, DLAT and SOD1 decreased after ES + Cu²⁺ treatment, but the addition of ATTM reversed the expression trend of these proteins ($P < 0.05$). **Conclusion** The combination of ES and Cu²⁺ can effectively inhibit the proliferation and migration of PLC/PRF/5 and Huh-7 of hepatocellular carcinoma cells, and induce Cuproptosis, which provides a new strategy for the treatment of hepatocellular carcinoma.

Key words Elesclomol-copper ion; hepatocellular carcinoma; cuproptosis; proliferation; migration

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intervention at 100 mg/kg and 200 mg/kg effectively reversed these changes, lowering MDA and NOX4 levels ($t = 3.35, 4.30, P < 0.05$; $t = 5.44, 7.44, P < 0.01$), while restoring SOD ($t_{200 \text{ mg/kg}} = 4.04, P < 0.05$) and GSH ($t = 4.70, P < 0.05$; $t = 5.50, P < 0.01$). Endoplasmic reticulum stress markers, including GRP94、GRP78、p-eIF2 α 、CHOP ($t = 3.31, 6.53, 5.18, 3.09; P < 0.05, 0.01, 0.01, 0.05$), were significantly upregulated in the DEX group compared to the normal group. However, TGP treatment at 100 mg/kg and 200 mg/kg dose-dependently suppressed the expression of GRP94、GRP78、p-eIF2 α 、CHOP ($t = 3.14, 4.95, 3.13, 4.25, P < 0.05; t = 4.03, 7.48, 4.68, 5.10, P < 0.01$) expression levels were significantly reduced compared to the DEX group, and there was no significant change in the TGP (50 mg/kg) treatment group. **Conclusion** TGP exerts protective effects against DEX-induced liver injury, and its mechanism is likely mediated by suppressing hepatic oxidative stress and endoplasmic reticulum stress triggered by DEX in rats.

Key words total glycosides of paeony; glucocorticoid; dexamethasone; liver injury; oxidative stress; endoplasmic reticulum stress

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