水苏碱调节 Hippo-YAP 信号通路对高糖诱导心肌细胞损伤模型影响的实验研究

宋彦庆，杜章辉，丁冰钰（山东中医药大学附属医院东营医院内分泌科，山东东营 257000）

摘 要：目的 研究水苏碱对高糖（HG）诱导的心肌细胞损伤的影响及其与 Hippo-Yes 相关蛋白（YAP）信号通路的调节作用。**方法** 以 30 mmol/L 葡萄糖对大鼠心肌细胞 H9C2 进行 HG 刺激以建立心肌细胞损伤模型，然后用 0.05，0.10，0.15，0.20 mmol/L 的水苏碱处理细胞并用 CCK-8 法检测其活性，筛选水苏碱的作用浓度。然后将 H9C2 细胞分为对照（ctrl）组、HG 组、低浓度水苏碱组、高浓度水苏碱组和高浓度水苏碱 +Hippo-YAP 信号通路抑制剂（TDI-011536）组。除 ctrl 组用 5 mmol/L 葡萄糖培养细胞外，其余 4 组均用 30 mmol/L 葡萄糖培养，低、高浓度水苏碱组分别用 0.05，0.10mmol/L 水苏碱培养 24h，高浓度水苏碱 +TDI-011536 组用 0.10 mmol/L 水苏碱和 3.00 μmol/L TDI-011536 培养 24h。CCK-8 法检测细胞增殖，流式细胞术检测细胞凋亡，ELISA 法检测丙二醛（MDA）和超氧化物歧化酶（SOD）水平，Western blot 检测磷酸化 YAP（p-YAP），YAP，增殖细胞核抗原（PCNA）和 B 淋巴细胞瘤 -2 基因（Bcl-2）蛋白水平。**结果** 与 ctrl 组比较，0.05，0.10，0.15，0.20 mmol/L 水苏碱处理 HG 诱导的 H9C2 细胞存活率显著增加，差异具有统计学意义（t=8.32 ~ 29.67，均 P<0.01），水苏碱的半数抑制浓度（IC50 值）约为 0.09 mmol/L，选择接近和低于 IC50 的 0.05 mmol/L 和 0.10 mmol/L 浓度作为后续实验的水苏碱浓度。与 ctrl 组比较，HG 组细胞存活率显著降低（t=44.32，11.04，P<0.01）；与 HG 组比较，低浓度水苏碱组、高浓度水苏碱组细胞存活率显著升高（t=10.06，21.66，均 P<0.01）；与高浓度水苏碱组比较，高浓度水苏碱 +TDI-011536 组细胞存活率显著下降（t=9.54，P<0.01），差异具有统计学意义。与 ctrl 组比较，HG 组细胞凋亡率显著升高（t=36.74，P<0.01）；与 HG 组比较，低浓度水苏碱组、高浓度水苏碱组细胞凋亡率显著下降（t=11.04，26.78，均 P<0.01）；与高浓度水苏碱组比较，高浓度水苏碱 +TDI-011536 组细胞凋亡率显著升高（t=9.96，均 P<0.01），差异具有统计学意义。与 ctrl 组比较，HG 组 SOD 水平显著降低，MDA 水平显著升高（t=18.85，29.12，均 P < 0.01）；与 HG 组比较，低浓度水苏碱组、高浓度水苏碱组 SOD 水平显著升高（t=6.59，9.86，均 P < 0.01），MDA 水平显著降低（t=13.45，23.36，均 P < 0.01）；与高浓度水苏碱组比较，高浓度水苏碱 +TDI-011536 组 SOD 水平显著降低，MDA 水平显著升高（t=5.30，6.98），差异具有统计学意义（均 P < 0.01）。与 ctrl 组比较，HG 组 p-YAP，p-YAP/YAP，PCNA，Bcl-2 蛋白水平显著降低，YAP 蛋白水平显著升高（t=15.36~45.00，均 P < 0.01）；与 HG 组比较，低浓度水苏碱组、高浓度水苏碱组的 p-YAP，p-YAP/YAP，PCNA，Bcl-2 蛋白水平显著升高，YAP 蛋白水平显著降低（t=5.51~25.15，均 P < 0.01）；与高浓度水苏碱组比较，高浓度水苏碱 +TDI-011536 组 p-YAP，p-YAP/YAP，PCNA，Bcl-2 蛋白水平显著降低，YAP 蛋白水平显著升高（t=4.27~11.25），差异具有统计学意义（均 P < 0.05）。**结论** 水苏碱可能通过激活 Hippo-YAP 信号通路抑制氧化应激和细胞凋亡，进而改善 HG 诱导的心肌细胞损伤。

关键词：水苏碱；Hippo-Yes 相关蛋白信号通路；高糖；心肌细胞；损伤
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Experimental Study on the Effect of Stachydrine on Myocardial Cell Injury Model Induced by High Glucose Through Regulating Hippo-YAP Signaling Pathway

SONG Yanqing, DU Zhanghui, DING Bingyu (*Department of Endocrinology, Dongying Hospital, Affiliated Hospital of Shandong University of Chinese Medicine, Shandong Dongying* 257000*, China*)

Abstract: Objective To study the effect of stachydrine on cardiomyocyte damage induced by high glucose (HG) and its regulation of Hippo-Yes-associated protein (YAP) signaling pathway. **Methods** Rat myocardial cells H9C2 were stimulated by HG with 30 mmol/L glucose to establish the myocardial cell injury model, and then treated with 0.05, 0.10, 0.15, 0.20 mmol/L of stachydrine and their activities were detected by CCK-8 method, and the action concentration of stachycarine was screened. Then H9C2 was grouped into control (ctrl) group, HG group, low concentration stachydrine group, high concentration stachydrine group, and high concentration stachydrine+Hippo-YAP signaling pathway inhibitor (TDI-011536) group. Except for the ctrl

作者简介：宋彦庆（1974−），男，硕士，副主任医师，研究方向：内分泌疾病，E-mail：fdfg_d2022@163.com。
通讯作者：丁冰钰，E-mail：xunxiannaoji303@163.com。

group cultured with 5 mmol/L glucose, the other 4 groups were cultured with 30 mmol/L glucose, and the low and high concentration stachydrine groups were cultured with 0.05 and 0.10 mmol/L threonine for 24h, respectively. The high concentration stachydrine +TDI-011536 group were cultured with 0.10 mmol/L stachydrine and 3.00 μmol/L TDI-011536 for 24h. CCK-8 method was applied to detect cell proliferation. Flow cytometry was applied to detect apoptosis. ELISA were applied to detect the level of malondialdehyde (MDA) and superoxide dismutase (SOD). Western blot was applied to detect the level of phosphorylated YAP (p-YAP), YAP, proliferating cell nuclear antigen (PCNA), and B-cell lymphoma-2 (Bcl-2) proteins. **Results** Compared with ctrl group, the survival rate of H9C2 cells induced by HG was significantly increased by 0.05, 0.10, 0.15, 0.20 mmol/L stachydrine treatment, and the differences were statistically significant (t=8.32~29.67,all P<0.01). The 50% inhibitory concentration (IC50) value of stachydrine was about 0.09 mmol/L, and the concentrations of 0.05 mmol /L and 0.10 mmol /L close to and lower than IC50 were selected as the concentrations of hydrostachyine for subsequent experiments. Compared with the ctrl group, the survival rate in HG group was significantly decreased (t=44.32, P<0.01). Compared with HG group, the cell survival rate of low concentration stachydrine group and high concentration stachydrine group was significantly increased (t=10.06, 21.66, all P<0.01). Compared with the high concentration stachydrine group, the cell survival rate in the high-concentration stachydrine +TDI-011536 group was significantly decreased (t=9.54, P<0.01) , and the differences were statistically significant, respectively. Compared with ctrl group, the apoptosis rate of HG group was significantly increased (t=36.74, P<0.01). Compared with HG group, the apoptosis rate of low concentration stachydrine group and high concentration stachydrine group was significantly decreased (t=11.04, 26.78, all P<0.01). Compared with the high concentration threonine group, the apoptosis rate of the high concentration stachydrine +TDI-011536 group was significantly increased (t=9.96, P<0.01), and the differences were statistically significant, respectively. Compared with ctrl group, SOD level in HG group was significantly decreased, MDA levels were significantly increased (t=18.85, 29.12, all P<0.01). Compared with HG group, SOD level were significantly increased in low concentration stachydrine groups and high concentration stachydrine groups(t=6.59,9.86,all P<0.01), MDA level were significantly decreased (t=13.45, 23.36, all P<0.01). Compared with the high concentration stachydrine group, the SOD level in the high concentration hydrostatin +TDI-011536 group was significantly decreased. MDA levels were significantly increased, and the differences were statistically significant (t=5.30, 6.98, all P<0.01), respectively. Compared with ctrl group,the level of p-YAP, p-YAP/YAP, PCNA, Bcl-2 protein were significantly decreased, and the level of YAP protein was significantly increased (t=15.36 ~ 45.00, all P<0.01). Compared with HG group, the level of p-YAP, p-YAP/YAP, PCNA, Bcl-2 protein were significantly increased in low concentration stachydrine group and high concentration stachydrine group, the level of YAP protein levels were significantly decreased (t=5.51 ~ 25.15, all P<0.01).Compared with the high concentration stachydrine group, the level of p-YAP, p-YAP/YAP, PCNA, Bcl-2 protein in the high concentration hydrothreonine +TDI-011536 group were significantly decreased, the level of YAP protein significantly increased, the differences were statistically significant (t=4.27 ~ 11.25, all P<0.05). **Conclusion** Stachydrine may inhibit oxidative stress and apoptosis by activating Hippo-YAP signaling pathway, thereby ameliorating HG-induced myocardial cell damage.

Keywords：stachydrine; Hippo-Yes-associated protein signaling pathway; high glucose; myocardial cells; injury

糖尿病性心肌病（diabetic cardiomyopathy，DC）是糖尿病引发的疾病，长期糖尿病易导致心脏功能变化进而发展为心力衰竭，目前还没有针对 DC 的特殊治疗方法[1-3]。故仍需探究 DC 的复杂机制并寻找有效的治疗药物。益母草是一种传统中药，具有抗炎、抗氧化、抗凋亡和治疗心血管疾病等作用[4]。水苏碱是提取自益母草的生物活性碱，可保护心脏、减轻心肌细胞肥大[5-6]。据报道，水苏碱通过调节 Hippo-Yes-associated protein（YAP）信号通路使新生大鼠免受缺氧缺血性脑损伤[7]，并通过 EPAC1/Rap1 信号通路抑制缺氧复氧诱导的心肌细胞凋亡[8]。但水苏碱在高糖（high glucose，HG）诱导的心肌细胞损伤中的作用尚未报道。Hippo-YAP 通路在心血管生长和心脏再生等过程中具有重要作用[9]。例如 Hippo-YAP 在心肌梗死疾病中是氧化应激和细胞凋亡的关键介质[10]；在高糖（HG）诱导的心脏成纤维细胞中 Hippo-YAP 受到 lncRNA 的调控并干预 YAP 的核易位，减轻 DC 小鼠炎症[11]。但水苏碱在 HG 诱导的心肌细胞损伤中的作用还不清楚。故本研究探讨水苏碱对 HG 诱导的心肌细胞损伤的影响和潜在机制，以期为水苏碱治疗 DC 的临床应用提供一定的理论依据。

1 材料与方法

1.1 细胞来源 大鼠心肌细胞 H9C2（南京安研生物科技有限公司）。

1.2 主要试剂与仪器 DMEM 培养液（批号 139555，成都博瑞特化学技术有限公司）；胎牛血清（FBS）（批号 WKQ-0004317，四川省维克奇生物科技

有限公司）；细胞计数试剂盒（cell counting kit-8，CCK-8，批号 CD28526，武汉纯度生物科技有限公司）；水苏碱（批号 FT-0777242，武汉丰泰威远科技有限公司）；Hippo-YAP 通路抑制剂 TDI-011536（批号 1210945-69-9，上海楼岚生物科技有限公司）；细胞凋亡试剂盒（批号 CSY6002S，武汉科斯坦生物科技有限公司）；大鼠超氧化物歧化酶（superoxide Dismutase，SOD）试剂盒（批号 EK-R30266，上海名劲生物科技有限公司）；大鼠丙二醛（malondialdehyde，MDA）试剂盒（批号 220305，上海桥杜生物科技有限公司）；二喹啉甲酸（bicinchoninic acid，BCA）蛋白浓度测定试剂盒（批号 PC0020，北京索莱宝科技有限公司）；兔源一抗磷酸化 YAP（phosphorylated YAP，p-YAP），YAP，增殖细胞核抗原（proliferating cell nuclear antigen，PCNA），β 微管蛋白（β-Tubulin），B 淋巴细胞瘤 -2 基因（B-cell lymphoma-2，Bcl-2），辣根过氧化物酶标记的山羊抗兔二抗 [批号 abs106569，abs133484，abs120180，abs131994，abs131701，abs20040，爱必信（上海）生物科技有限公司]。Spark 多功能酶标仪（西安纵横仪器科技有限公司）；BD FACSCalibur 流式细胞仪，ChemiDocT-MXRS+ 扫描成像仪（美国 BD 公司）。

1.3 方法

1.3.1 细胞培养：H9C2 细胞在添加 10ml/dl FBS 和 1g/dl 青霉素和链霉素的 DMEM 培养液中，5%（v/v）CO_2，37℃培养箱中培养，当细胞密度达到 80%~90% 时，开始后续实验。

1.3.2 水苏碱浓度筛选及细胞分组：将达到实验条件的 H9C2 细胞以 4×10^3 个 /ml 的密度接种在 96 孔板中。然后加入 30 mmol/L 葡萄糖培养 24 h 诱导损伤[12]，再用不同剂量（0.05，0.10，0.15，0.20 mmol/L）的水苏碱[13]处理细胞，对照（ctrl）组细胞不加入水苏碱。24 h 后，每孔加入 10 μl CCK-8 试剂，37℃孵育 3 h，用多功能酶标仪测定 450 nm 处的吸收度（*A* 值），根据公式：细胞存活率 =（$A_{实验组}-A_{空白组}$）/（$A_{对照组}-A_{空白组}$）×100%，计算细胞存活率和半数抑制浓度（IC50）值。

依据上述结果将 HG 诱导的 H9C2 细胞分为 HG 组、低浓度水苏碱组、高浓度水苏碱组、高浓度水苏碱 +TDI-011536 组。低浓度水苏碱组、高浓度水苏碱组分别用 0.05mmol/L，0.10mmol/L 水苏碱培养 24h；高浓度水苏碱 +TDI-011536 组用 0.10 mmol/L 水苏碱和 3.00 μmol/L TDI-011536 培养 24[14]。用 5 mmol/L 葡萄糖培养的细胞作为 ctrl 组。

1.3.3 CCK-8 法：将各组 H9C2 以 4×10^3 个 /ml 的浓度接种到 96 孔板中，采用 1.3.1 中的方法监测细胞 *A* 值，根据公式计算各组细胞存活率。

1.3.4 流式细胞术：细胞用胰蛋白酶消化，PBS 清洗，加入 AnnexinV-FITC 和碘化丙啶（propidium iodide，PI）避光孵育细胞，采用 BD FACSCalibur 流式细胞仪分析细胞凋亡率。

1.3.5 ELISA 法：收集各组细胞，3 000 r/min 离心 20 min，收集细胞上清液，采用 ELISA 法分别测定 450 nm 的 *A* 值，并根据标准曲线计算各组 H9C2 细胞中 SOD 和 MDA 的含量。

1.3.6 Western blot 分析：H9C2 细胞用 RIPA 蛋白裂解缓冲液分解，再加入蛋白酶抑制剂提取总蛋白。用 BCA 检测试剂盒进行浓度测定，SDS-PAGE 分离蛋白，并转移到硝酸纤维素膜中，室温下用 5g/dl 脱脂牛奶封闭 1h，然后用一抗 p-YAP（1∶2 000），YAP（1∶2 000），PCNA（1∶1 000），Bcl-2（1∶1 000），β-Tubulin（1∶1 000）在 4℃孵育过夜，室温下用辣根过氧化物酶（HRP）标记的二抗孵育膜 1 h。加入 ECL 试剂，在扫描成像仪中检测蛋白质条带，利用 Image Lab™ 软件进行图像分析。

1.4 统计学分析 使用 SPASS 25.0 软件进行统计分析，计量数据采用平均值 ± 标准差（$\bar{x}\pm s$）表示。采用单因素方差分析比较多组差异，采用 Tukey 检验进行两两比较，$P<0.05$ 为差异具有统计学意义。

2 结果

2.1 水苏碱对 HG 诱导的 H9C2 细胞活性的影响 CCK-8 检测结果显示，与 ctrl 组比较，0.05，0.10，0.15，0.20 mmol/L 水苏碱分别处理的 HG 诱导 H9C2 细胞的存活率（38.75%±3.64%，48.66%±4.57%，65.08%±3.91%，74.87%±4.62% vs 24.68%±3.88%）显著增加，差异具有统计学意义（t=8.32，14.18，23.89，29.67，all $P<0.01$）。线性趋势检验结果显示，r^2=0.952，F=558.263，$P<0.01$，说明水苏碱浓度与细胞存活率差异显著。水苏碱的 IC50 值约为 0.09 mmol/L，所以选接近 IC50 的 0.10 mmol/L 浓度水苏碱处理细胞作为高浓度水苏碱组，选低于 IC50 的 0.05 mmol/L 浓度的水苏碱处理细胞作为低浓度水苏碱组，并以此结果进行后续分组及功能验证实验。

2.2 水苏碱对 HG 诱导的 H9C2 细胞增殖和凋亡的影响 见表 1。与 ctrl 组比较，HG 组细胞存活率显著降低，凋亡率显著升高，差异具有统计学意义（t=44.32，36.74，均 $P<0.01$）；与 HG 组比较，低浓度水苏碱组、高浓度水苏碱组细胞存活率显著上升，凋亡率显著下降，差异具有统计学意义（t=10.04，21.66；11.04，26.78，均 $P<0.01$）；与高浓度水苏碱组比较，高浓度水苏碱 +TDI-011536 组细胞存活率显著下降，凋亡率显著升高，差异具有统计学意义（t=9.54，9.96，均 $P<0.01$）。以上结果表明，水苏碱可抑制 HG 诱导的 H9C2 细胞凋亡，并促进增殖。

表 1 水苏碱对 HG 诱导的 H9C2 细胞增殖、凋亡的影响（n=6，$\bar{x}\pm s$，%）

项目	ctrl 组	HG 组	低浓度水苏碱组	高浓度水苏碱组	高浓度水苏碱 +TDI-011536 组	F	P
细胞存活率	95.53 ± 4.45	26.06 ± 3.31	41.79 ± 3.29	56.44 ± 3.66	44.86 ± 4.19	283.73	<0.01
细胞凋亡率	8.83 ± 1.59	38.44 ± 2.29	29.54 ± 2.11	16.86 ± 1.95	24.89 ± 1.86	200.41	<0.01

2.3 水苏碱对 HG 诱导的 H9C2 细胞氧化应激标志物的影响 见表 2。与 ctrl 组比较，HG 组 SOD 水平显著降低，MDA 水平显著升高，差异具有统计学意义（t=18.85，29.12，均 $P<0.01$）；与 HG 组比较，低浓度水苏碱组、高浓度水苏碱组 SOD 水平显著升高，MDA 水平显著降低，差异具有统计学意义（t=6.59，13.45；9.86，23.36，均 $P<0.01$）；与高浓度水苏碱组比较，高浓度水苏碱 +TDI-011536 组 SOD 水平显著降低，MDA 水平显著升高，差异具有统计学意义（t=5.30，6.98，均 $P<0.01$）。以上结果表明，水苏碱可通过调节 SOD，MDA 水平抑制 HG 诱导的 H9C2 细胞氧化应激。

表 2 水苏碱对 HG 诱导的 H9C2 细胞氧化应激标志物的影响（n=6，$\bar{x}\pm s$，nmol/mg）

项目	ctrl 组	HG 组	低浓度水苏碱组	高浓度水苏碱组	高浓度水苏碱 +TDI-011536 组	F	P
SOD	28.97 ± 1.96	14.82 ± 1.58	19.77 ± 1.64	24.92 ± 1.49	20.94 ± 2.38	50.86	<0.01
MDA	0.92 ± 0.13	2.84 ± 0.19	2.19 ± 0.15	1.30 ± 0.15	1.76 ± 0.18	129.97	<0.01

2.4 水苏碱对 HG 诱导的 H9C2 细胞中 p-YAP，YAP，PCNA，Bcl-2 蛋白表达的影响 见表 3。与 ctrl 组比较，HG 组 p-YAP，p-YAP/YAP，PCNA，Bcl-2 蛋白水平显著降低，YAP 蛋白水平显著升高，差异具有统计学意义（t=21.00，45.00，27.94，15.36，21.10，均 $P<0.01$）；与 HG 组比较，低浓度水苏碱组、高浓度水苏碱组的 p-YAP，p-YAP/YAP，PCNA，Bcl-2 蛋白水平显著升高，YAP 蛋白水平显著降低，差异具有统计学意义（t=7.82，15.64；10.26，25.15；10.67，20.83；5.51，10.73；11.38，16.83，均 $P<0.01$）；与高浓度水苏碱组比较，高浓度水苏碱 +TDI-011536 组 p-YAP，p-YAP/YAP，PCNA，Bcl-2 蛋白水平显著降低，YAP 蛋白水平显著升高，差异具有统计学意义（t=4.94，11.25，6.35，4.64，4.27，均 $P<0.05$）。以上结果表明，水苏碱可通过上调 YAP 磷酸化水平激活 HG 诱导的 H9C2 细胞中 Hippo-YAP 信号通路。

表 3 水苏碱对 HG 诱导的 H9C2 细胞中 p-YAP，YAP，PCNA，Bcl-2 蛋白表达的影响（n=6，$\bar{x}\pm s$）

项目	ctrl 组	HG 组	低浓度水苏碱组	高浓度水苏碱组	高浓度水苏碱 +TDI-011536 组	F	P
p-YAP	0.75 ± 0.08	0.24 ± 0.06	0.43 ± 0.05	0.62 ± 0.04	0.50 ± 0.06	63.17	<0.01
YAP	0.49 ± 0.07	1.38 ± 0.12	0.90 ± 0.14	0.67 ± 0.09	0.85 ± 0.08	62.51	<0.01
p-YAP/YAP	1.53 ± 0.11	0.17 ± 0.02	0.48 ± 0.06	0.93 ± 0.08	0.59 ± 0.07	294.31	<0.01
PCNA	1.31 ± 0.12	0.21 ± 0.06	0.63 ± 0.11	1.03 ± 0.08	0.78 ± 0.10	111.30	<0.01
Bcl-2	0.95 ± 0.11	0.42 ± 0.05	0.61 ± 0.09	0.79 ± 0.09	0.63 ± 0.07	33.61	<0.01

3 讨论

DC 是糖尿病引发的心肌特异性微血管并发症，会增加心脏衰竭 [15]。DC 的发病机制复杂，仍未研究透彻，且目前临床上的有效治疗方法较少，预后也较差 [15-16]。HG 引起的葡萄糖代谢异常是 DC 的关键起始因素 [17]。故本研究采用 HG 处理 H9C2 建立 DC 体外细胞损伤模型，结果显示，HG 处理后的细胞增殖能力下降，凋亡率增高，氧化应激指标（SOD，MDA）变化明显，而且增殖、凋亡相关蛋白 PCNA，Bcl-2 表达下降，与既往研究一致 [18-19]，表明 HG 诱导了大鼠心肌细胞损伤，提示细胞模型的成功构建。

水苏碱具有治疗纤维化、心血管疾病、炎症等作用 [20]。水苏碱可以促进血管生成，减少苯肾上腺素诱导的心肌肥厚并改善心功能 [21-22]。本研究发现，水苏碱可促进 HG 诱导的 H9C2 增殖，并减少细胞凋亡，减轻氧化应激反应，且高剂量的效果更佳。提示水苏碱可通过增强心肌细胞增殖、抑制凋亡来减轻 HG 诱导的心肌细胞损伤，这与水苏碱在缺氧 / 复氧诱导的 H9C2 细胞中的作用 [23] 一致，说明水苏碱可能对 DC，心肌缺血等疾病具有一定的治疗作用。推测其原因可能是水苏碱通过改善微血管生成来增强细胞的增殖能力、抑制心肌细胞损伤来改善心力衰竭从而达到治疗 DC 的目的。

Hippo-YAP 信号通路属于核心激酶级联通路，其激活时 YAP 磷酸化水平增高，入核 YAP 减少，

从而调节下游基因 cyclin D1 等的表达，最终达到调控细胞增殖、凋亡的作用[24-25]。研究显示，抑制 Hippo-YAP 通路可促进心肌细胞增殖，改善小鼠心脏衰竭[26]。在 DC 中，Hippo-YAP 通路失调，YAP 活性增加，导致心肌细胞生长异常[27-28]。本研究发现，HG 组 p-YAP 蛋白表达减少，YAP 蛋白表达升高，说明 Hippo-YAP 通路在 DC 进展中处于阻滞状态；而低、高浓度水苏碱处理可增加 p-YAP 蛋白表达，减少 YAP 蛋白表达，推测水苏碱可能通过激活 Hippo-YAP 信号通路降低 HG 诱导 H9C2 细胞损伤。为验证上述猜想，本研究用 Hippo-YAP 通路抑制剂 TDI-011536 和高浓度水苏碱联合干预 HG 诱导的 H9C2 细胞，结果发现，TDI-011536 减弱了高浓度水苏碱对 HG 诱导的 H9C2 损伤的改善作用，证实了水苏碱可能通过激活 Hippo-YAP 信号通路抑制氧化应激和凋亡的发生，进而减轻 HG 诱导的 H9C2 细胞损伤。

综上，水苏碱可能通过激活 Hippo-YAP 信号通路减轻细胞凋亡和氧化应激，进而缓解 HG 诱导的心肌细胞损伤，为治疗提供了一定的参考价值。但是本研究缺乏体内实验研究，且回复实验结果提示可能有别的通路介导水苏碱缓解心肌细胞损伤的过程，后续会进行深入探究。

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