

·基础研究·

肥大细胞激活促进酒精性心肌病的心肌重构

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摘要:【目的】探讨肥大细胞(MC)在酒精性心肌病(ACM)中的作用及相关机制。【方法】采用酒精液体饲料喂养C57BL/6小鼠建立酒精性心肌病模型,部分模型小鼠给予肥大细胞稳定剂色甘酸钠干预。通过超声心动图、组织染色和Western blot评估心功能、心肌重构及相关蛋白表达。体外实验使用肥大细胞颗粒(MCGs)和/或酒精刺激乳大鼠心肌细胞,并应用通路抑制剂探讨其机制。【结果】长期酒精喂养可导致小鼠出现显著的心脏功能障碍,并激活肥大细胞,同时心肌组织中糜蛋白酶(chymase)、NOX2、NOX4及凋亡相关蛋白c-Caspase3表达升高($P<0.05$)。色甘酸钠干预可改善心功能,抑制肥大细胞激活,并降低上述蛋白表达。体外实验显示,MCGs或酒精单独处理均可上调心肌细胞c-Caspase3、NOX2、NOX4和磷酸化PKC β 1(p-PKC β 1)的表达,两者联合刺激具有协同效应。蛋白酶激活受体2(PAR2)抑制剂可下调MCGs诱导的上述蛋白表达。PKC β 1抑制剂亦能抑制MCGs诱导的c-Caspase3、NOX2和NOX4表达。糜蛋白酶抑制剂对MCGs诱导的心肌细胞凋亡具有部分抑制作用。【结论】在酒精性心肌病发生过程中,肥大细胞被激活,其释放的颗粒物质可能通过PAR2-PKC β 1-NOX2/NOX4信号通路促进心肌细胞凋亡,糜蛋白酶在此过程中发挥重要作用。

关键词:肥大细胞;酒精性心肌病;心肌重构;糜蛋白酶;细胞凋亡

中图分类号:R542.2

文献标志码:A

文章编号:1672-3554(2026)03-0451-08

DOI:10.11714/jysu.med.YX20260025

Mast Cell Activation Promotes Myocardial Remodeling in Alcoholic Cardiomyopathy

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Abstract: 【Objective】 To investigate the role and related mechanisms of mast cells (MCs) in alcoholic cardiomyopathy (ACM). 【Methods】 An ACM model was established in C57BL/6 mice by feeding them with alcohol liquid diet, and some model mice were intervened with cromolyn sodium, a mast cell stabilizer. Cardiac function, myocardial remodeling and the expression of related proteins were evaluated by echocardiography, tissue staining and Western blot. In vitro, neonatal rat ventricular cardiomyocytes (NRVCs) were stimulated with mast cell granules (MCGs) and/or alcohol, and pathway inhibitors were used to explore the underlying mechanisms. 【Results】 Long-term alcohol feeding caused

收稿日期:2026-02-11

录用日期:2026-04-07

基金项目:国家自然科学基金(81370284)

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significant cardiac dysfunction and mast cells activation in mice, accompanied by increased expressions of chymase, NOX2, NOX4 and apoptosis-related protein cleaved Caspase-3 (c-Caspase3) in myocardial tissues ($P<0.05$). Pretreatment with cromolyn sodium improved cardiac function, inhibited mast cell activation, and reduced the expressions of the above proteins. In vitro experiments showed that treatment with MCGs or alcohol alone up-regulated the expressions of c-Caspase3, NOX2, NOX4 and phosphorylated PKC β 1 (p-PKC β 1) in cardiomyocytes, and the combined stimulation of the two had a synergistic effect. Protease-activated receptor 2 (PAR2) inhibitor down-regulated the MCG-induced expressions of the above proteins. PKC β 1 inhibitor also inhibited the MCG-induced expressions of c-Caspase3, NOX2 and NOX4. Chymase inhibitor exerted a partial inhibitory effect on MCG-induced cardiomyocyte apoptosis.【Conclusion】 During the development of ACM, mast cells are activated, and the released MCGs may promote cardiomyocyte apoptosis through the PAR2-PKC β 1-NOX2/NOX4 signaling pathway, in which chymase plays an important role.

Key words: mast cell; alcoholic cardiomyopathy; cardiac remodeling; chymase; apoptosis

[J SUN Yat-sen Univ(Med Sci), 2026, 47(3):451-458]

酒精性心肌病(alcoholic cardiomyopathy, ACM)是由于长期过量饮酒所致的一种非缺血性扩张型心肌病,约占所有不明原因扩张型心肌病的3.8%~47%^[1]。研究表明,经济增长与饮酒人群扩大呈正相关^[2],提示未来酒精性心肌病患者可能会相应增多。该病与其他类型扩张型心肌病相似,其特征包括左心室扩张、收缩功能受损、心室壁变薄、细胞内细胞器结构破坏、收缩蛋白丢失以及钙稳态失调等^[3]。细胞凋亡在酒精性心肌病发生发展中起关键作用^[4],已有研究发现,肥大细胞(mast cell, MC)来源的糜蛋白酶(chymase)可直接诱导心肌细胞凋亡^[5],然而肥大细胞及其炎症介质在酒精性心肌病中的作用尚未明确。肥大细胞起源于骨髓CD34⁺多能造血干细胞,以前体细胞形式进入循环,迁移至各种组织并在局部微环境中分化成熟^[6]。肥大细胞内储存多种炎症介质,包括组胺、蛋白酶(如糜蛋白酶)、细胞因子等,在肥大细胞被激活后释放并发挥多种生物学效应。以往研究主要关注肥大细胞在先天免疫及过敏反应中的作用,近几十年人们发现其与多种心血管疾病相关。特发性扩张型心肌病(idiopathic dilated cardiomyopathy, iDCM)和缺血性心肌病(ischemic cardiomyopathy, ICM)患者的心肌组织中肥大细胞数量显著增多^[7];此外,特发性扩张型心肌病心脏组织中肥大细胞数量的增加与胶原沉积程度相关,且其中较多肥大细胞含有糜蛋白酶^[8]。糜蛋白酶是主要来源于肥大细胞的丝氨酸蛋白酶,可通过多种机制影响心肌重构。它可直接诱导心肌细胞凋亡^[5]、激活参与细胞外基质降解的基质金属蛋白酶(matrix metalloproteinase 2,

MMP-2; matrix metalloproteinase 9, MMP-9)、促进I型胶原前体转化为I型胶原、激活转化生长因子- β 1(transforming growth factor-beta 1, TGF- β 1)、不依赖血管紧张素转换酶而参与血管紧张素II的生成^[9],而血管紧张素II被公认具有较强促炎和促纤维化作用^[10]。鉴于肥大细胞及糜蛋白酶在特发性扩张型心肌病的心肌重构过程中发挥重要作用,而酒精性心肌病与特发性扩张型心肌病在病理特征上具有诸多相似性,本研究拟探讨肥大细胞及其炎症因子在酒精性心肌病中的作用与潜在机制。

1 材料与方法

1.1 动物实验

8周龄雄性C57BL/6小鼠购自北京维通利华实验动物技术有限公司。适应1周后,小鼠被随机分成2组,分别给予含酒精的Lieber-DeCarli液体饲料(南通特洛菲饲料科技有限公司)或等热量对照液体饲料喂养12周。酒精含量在前8周为4%(体积/质量),后4周提高至5%。酒精喂养小鼠进一步分为3组:酒精喂养组(Alcohol)、酒精喂养+腹腔注射PBS组(Alcohol + PBS)、酒精喂养+肥大细胞稳定剂色甘酸钠干预组(Alcohol + CRO,后8周色甘酸钠40 mg/kg每日腹腔注射^[11])。对照组(CON)全程给予等热量液体饲料。所有涉及动物的实验流程均获得中山大学动物伦理与福利委员会的批准(动物实验伦理批件号为:SYSU-IACUC-2019-000223)。

1.2 超声心动图检查

使用配备30-MHz探头的高分辨率小动物超声Vevo 2100系统无创评估心功能。小鼠经体积分数4%异氟烷麻醉后维持于麻醉状态。测量指标包括左心室舒张末期内径(left ventricular end-diastolic diameter, LVEDD)、左心室收缩末期内径(left ventricular end-systolic diameter, LVESD)、舒张期左心室后壁厚度(LV posterior wall thickness in diastolic, LVPW.d)、收缩期左心室后壁厚度(LVPW in systolic, LVPW.s)、射血分数(ejection fraction, EF,%)及左室短轴缩短率(fractional shortening, FS,%)。

1.3 组织学分析

小鼠深度麻醉后经心脏穿刺放血处死。取右心室血样,离心($150 \times g$, 10 min)后取上清储存于 -80°C 。心脏取出后于冰预冷PBS中冲洗,部分用40 g/L多聚甲醛固定并石蜡包埋,部分冻存于 -80°C 。石蜡切片行Masson染色观察胶原沉积,甲苯胺蓝(Toluidine blue)染色显示肥大细胞。切片于奥林巴斯BX51光学显微镜下观察并采集图像。

1.4 细胞培养

参照文献方法^[12]从成年SD大鼠腹腔分离肥大细胞(peritoneal mast cells, PMCs)。大鼠经深度麻醉后采用颈椎脱臼法处死,腹腔注入预冷HBSS溶液,轻柔按摩腹腔后收集灌洗液,离心获得细胞沉淀。若细胞沉淀中混有红细胞,则加入0.9 mL无菌超纯水低渗裂解红细胞,随后迅速加入0.1 mL $10 \times$ PBS以恢复等渗。以 $150 \times g$ 离心5 min,弃上清,将细胞沉淀重悬于体积分数70% Percoll溶液(GE Healthcare)。在重悬液上方缓慢加入HBSS溶液形成密度梯度,再次离心($150 \times g$, 10 min)后收集细胞沉淀。甲苯胺蓝染色鉴定纯度 $\geq 90\%$,将符合纯度的肥大细胞重悬于DMEM/F12培养基中。为获得肥大细胞颗粒(MC granules, MCGs),用C48/80 (Sigma Aldrich)刺激PMCs^[5],收集含分泌颗粒的上清作为100%MCGs,分装冻存于 -80°C 。

参照改良酶消化法^[13]从1~2日龄SD大鼠分离乳大鼠心肌细胞(neonatal rat ventricular cardiomyocytes, NRVCs)。首先在无菌条件下迅速取出乳鼠心脏,仔细分离左心室组织,将其剪成约 1 mm^3 的小块,并在 37°C 下用质量分数0.125%胰蛋白酶进行序贯消化。消化后的组织经离心后弃上清,沉淀重悬于质量分数0.05% I型胶原酶中,继续于 37°C 长时间消化,随后加入等体积含体积分

数10%新生牛血清的DMEM培养基以终止消化。收集细胞悬液,离心后弃上清,沉淀重悬于含体积分数10% NBS的DMEM培养基中。为减少成纤维细胞污染,将细胞悬液置于 37°C 预铺板1 h,促使成纤维细胞优先贴壁。收集未贴壁的心肌细胞,以 0.8×10^6 /孔的密度接种于六孔板中,采用含体积分数10% NBS和0.1 mmol/L BrdU的DMEM/F12培养基进行培养,以抑制非心肌细胞增殖。培养48 h后,进行24 h血清饥饿处理,随后用于后续实验。

1.5 TUNEL检测

使用TUNEL细胞凋亡检测试剂盒(Yeasen)检测组织切片及NRVCs的凋亡情况。组织切片脱蜡复水后经蛋白酶K处理,与TUNEL反应液孵育,并用过氧化物酶和DAB显色。NRVCs经40 g/L多聚甲醛固定后行TUNEL染色,DAPI复染核。使用光学显微镜(组织)或荧光显微镜(细胞)采集图像。

1.6 蛋白免疫印迹

使用含蛋白酶及磷酸酶抑制剂的RIPA裂解液提取组织或细胞蛋白,BCA法测定蛋白浓度。等量蛋白经质量分数10% SDS-PAGE分离后转印至PVDF膜,质量分数5%脱脂牛奶封闭, 4°C 孵育一抗过夜:Chymase (Proteintech)、NOX2 (nicotinamide adenine dinucleotide phosphate oxidases 2, Proteintech)、NOX4 (nicotinamide adenine dinucleotide phosphate oxidases 4, Proteintech)、p-PKC β 1 (Abcam)、cleaved-Caspase3 (c-Caspase3, Cell Signaling Technology)或GAPDH (Servicebio)。洗膜后孵育相应二抗,使用增强化学发光检测系统显示蛋白条带。用Quantity One软件对条带强度进行光密度定量,以GAPDH为内参。

1.7 统计分析

数据以均值 $\pm$ 标准误($\bar{x} \pm s$)或中位数与四分位数 $M(P_{25} \sim P_{75})$ 表示。所有实验独立重复至少3次。使用SPSS 29.0软件分析数据。多组间的均数经正态分布及方差齐性检验后使用单因素方差分析(one-way ANOVA),不适用方差分析的采用Kruskal-Wallis H 检验进行分析。组间两两比较采用Bonferroni法。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 色甘酸钠改善酒精所致小鼠心脏功能障碍

与对照组相比,酒精喂养12周对各组小鼠体质量及心脏/体质量比无明显影响(表1)。超声心

动图显示,酒精喂养小鼠心功能显著受损,表现为EF和FS降低;色甘酸钠干预可减轻上述损伤。酒精喂养小鼠LVESD显著增加,色甘酸钠处理可使其减小。酒精喂养小鼠LVEDD有增加的趋势,色甘酸钠干预可部分改善,但差异无统计学意义。

表1 各组小鼠基本特征、超声心动图结果、肥大细胞数量与脱颗粒率对比						
Table 1 Biometric, echocardiographic, and MC morphometric parameters of mice in different group						
[($\bar{x} \pm s$), $M(P_{25}-P_{75})$]						
Item	CON (n=8)	Alcohol (n=8)	Alcohol+PBS (n=6)	Alcohol+CRO (n=7)	F/H	P
Body weight/g	30.72±0.90	31.51±0.56	30.77±1.01	28.70±0.77	2.223	0.110
Heart/body weight/(mg/g)	5.50 (4.84–6.13)	4.71 (4.47–5.15)	4.72 (4.54–5.07)	4.90 (4.74–5.42)	4.754	0.191
EF/%	59.41 (58.74–63.00)	50.51 (49.09–51.69)*	54.64 (50.27–55.99)	58.22 (57.86–58.37) [#]	13.257	0.004
FS/%	30.68 (29.98–32.85)	25.24 (24.18–25.72)*	27.26 (24.99–28.46)	29.79 (29.59–29.91) [#]	12.640	0.005
LVEDD/mm	3.11 (2.84–3.40)	3.54 (3.30–3.87)	3.49 (3.34–3.63)	3.32 (3.09–3.38)	5.890	0.117
LVESD/mm	2.15±0.12	2.68±0.11*	2.55±0.09	2.29±0.06	6.271	0.008
LVPW.d/mm	0.87 (0.82–0.92)	0.77 (0.76–0.83)*	0.83 (0.80–0.94)	0.78 (0.77–0.80)	8.537	0.036
LVPW.s/mm	1.17 (1.06–1.22)	1.07 (1.03–1.11)	1.02 (0.93–1.29)	1.06 (1.00–1.17)	2.680	0.444
LV MCs number per section	7.50 (5.50–8.75)	7.50 (3.75–9.75)	6.00 (6.00–7.50)	5.00 (3.25–9.00)	1.012	0.798
MCs degranulation/%	46.43 (40.71–54.17)	100.00 (92.50–100.00)*	91.67 (83.33–100.00)	55.00 (12.50–65.00)	12.019	0.007

CON: control diet; CRO: cromolyn sodium; EF: ejection fraction; FS: fractional shortening; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LVPW.d: LV posterior wall thickness in diastole; LVPW.s: LV posterior wall thickness in systole; MC: mast cell. Data are presented as mean ± SEM, or median and quartile. One-Way ANOVA or Kruskal-Wallis *H* was used to test the difference between groups. **P* < 0.05 vs. CON group; [#]*P* < 0.05 vs. Alcohol group.

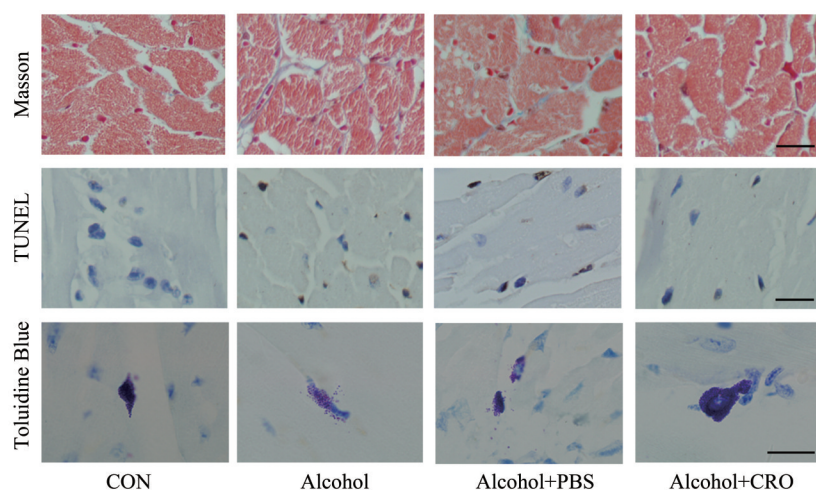
2.2 色甘酸钠可抑制酒精所致小鼠心肌重构、细胞凋亡及肥大细胞激活

Masson 染色结果显示,与对照组相比,长期酒精喂养可显著增加小鼠心肌组织的胶原沉积。此类结构异常伴随心肌细胞凋亡的明显增多。使用肥大细胞稳定剂色甘酸钠干预后,酒精引起的胶原积累与细胞凋亡均得到显著抑制(图1)。

尽管各组小鼠心肌中肥大细胞数量无显著差异,但酒精喂养组的肥大细胞脱颗粒率显著高于对照组(表1),而色甘酸钠处理可有效抑制这一现

象。如图1所示,酒精喂养组中可见处于激活状态的肥大细胞,其周围分布较多释放的颗粒;相比之下,对照组与色甘酸钠处理组则以静息态的肥大细胞为主。PBS处理对上述指标无影响。

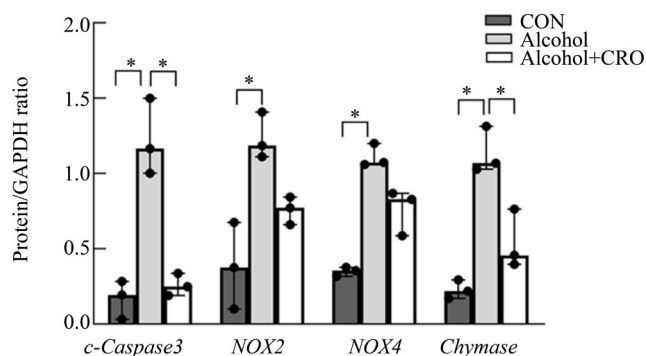
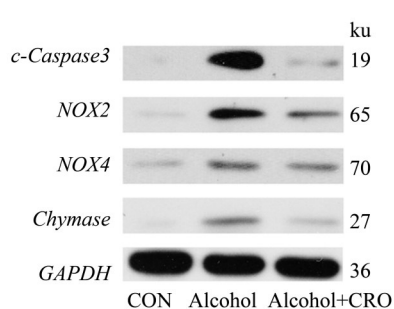
进一步研究表明,酒精喂养12周后,c-Caspase3表达显著上调(*P*<0.05),而色甘酸钠处理可部分逆转该变化(*P*<0.05)。同样,酒精诱导的NOX2和NOX4蛋白表达升高也在色甘酸钠干预后受到抑制。酒精喂养小鼠心肌组织中Chymase水平也显著升高(*P*<0.05),而色甘酸钠处理可降低其表达(图2)。



Representative images of heart sections from the indicated experimental groups stained for collagen deposition (Masson's trichrome), apoptosis (TUNEL) and MCs (toluidine blue). Scale bar=20 μ m.

图1 心肌组织 Masson、TUNEL 及甲苯胺蓝染色结果

Fig. 1 Masson, TUNEL and toluidine blue staining of myocardial tissues



Protein levels of c-Caspase3, NOX2, NOX4, and chymase in heart tissues from the indicated experimental groups. GAPDH serves as a loading control. Data are expressed as median and scatter dot plot; $n=3$. One-Way ANOVA or Kruskal-Wallis H was used to test the difference between groups (c-Caspase3: $F=35.438$, $P=0.000$; NOX2: $F=14.266$, $P=0.005$; NOX4: $H=10.385$, $P=0.016$; Chymase: $F=28.992$, $P=0.001$). * $P < 0.05$.

图2 心肌组织中 c-Caspase3、NOX2、NOX4 及 Chymase 的表达

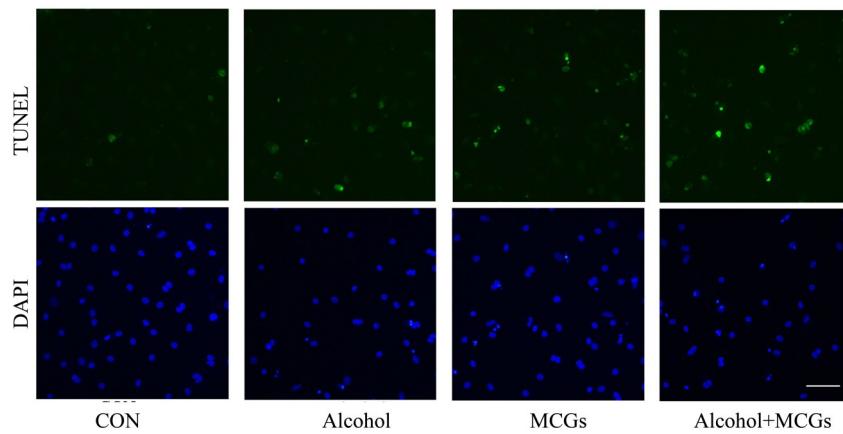
Fig. 2 Expressions of c-Caspase3, NOX2, NOX4 and Chymase in myocardial tissues

2.3 肥大细胞颗粒通过 PAR2-PKC β 1-NOX2/NOX4 途径加剧酒精诱导的心肌细胞凋亡

动物实验结果表明,肥大细胞活化与酒精性心肌病的心肌重构密切相关。为进一步探究其直接作用,我们收集 MCGs 并观察其对乳大鼠心肌细胞的影响。结果显示,单独使用酒精或 MCGs 处理均能诱导心肌细胞凋亡,而两者联合刺激则进一步加剧凋亡程度(图 3)。与此一致,酒精或 MCGs 单独处理均可上调乳大鼠心肌细胞中 c-Caspase3、NOX2 和 NOX4 的表达,且联合处理使这些蛋白的表达进一步升高(图 4A)。此外,本研究也发现,酒精或 MCGs 处理均能增强乳大鼠心肌细胞

PKC β 1 磷酸化,联合刺激则显著放大该效应(图 4A)。

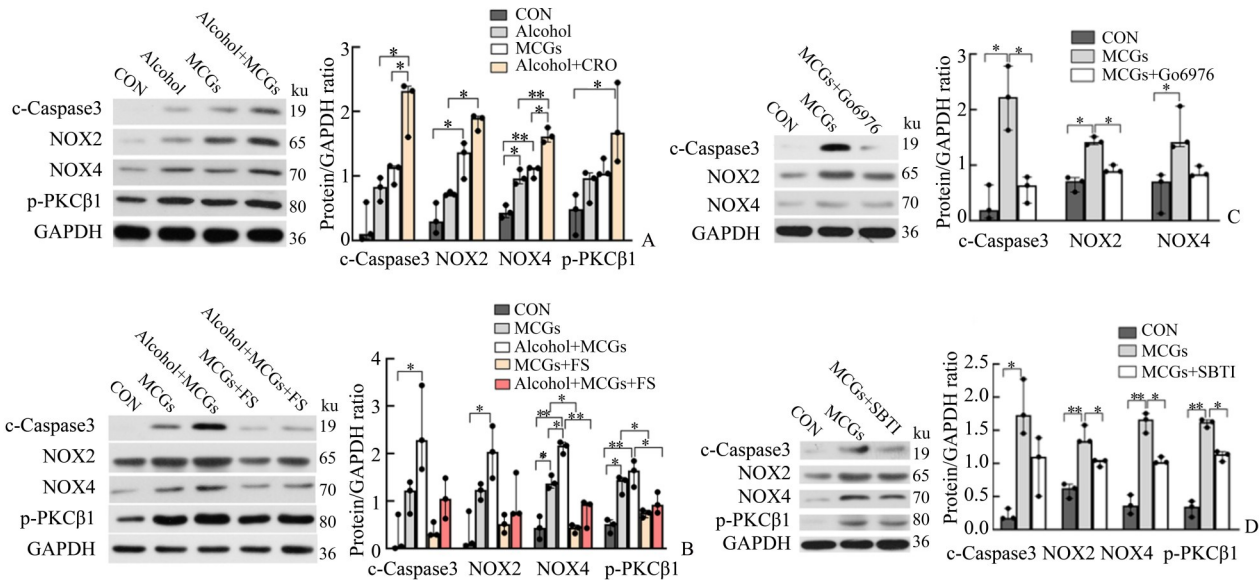
在此基础上,我们使用蛋白酶激活受体 2 (protease activated receptor-2, PAR2) 抑制剂 FSLRY-NH₂(FS) 进行干预,发现其可显著抑制 MCGs 单独或与酒精共同刺激所引起的 c-Caspase3、NOX2、NOX4 及 p-PKC β 1 上调(图 4B)。类似地,采用蛋白激酶 C β 1 (protein kinase C beta 1, PKC β 1) 抑制剂 G6976 预处理心肌细胞,也可有效抑制 MCGs 诱导的 c-Caspase3、NOX2 与 NOX4 表达升高,提示 PKC β 1 参与 MCGs 引起的心肌细胞凋亡(图 4C)。与此同时,使用 Chymase



Representative TUNEL staining (green) showing apoptosis in NRVCs treated with alcohol (100 mmol/L), MCGs (50% volume), or their combination for 24 h. Nuclei were counterstained with DAPI (blue). Scale bar=50 μ m.

图3 不同刺激下乳大鼠心肌细胞的凋亡情况

Fig. 3 Apoptosis of neonatal rat ventricular cardiomyocytes under different stimulations



A: Protein levels of c-Caspase3, NOX2, NOX4, and p-PKC β 1 in NRVCs treated with alcohol (100 mmol/L), MCGs (50% volume), or both for 24 h. B-D: Effects of pharmacological inhibition of PAR2 (FS, 200 μ mol/L) (B), PKC β 1 (Go6976, 1 μ mol/L) (C), or chymase (SBTI, 100 μ g/mL) (D) on protein expression in NRVCs challenged with MCGs and/or alcohol. FS or Go6976 was preincubated with NRVCs for 30 min prior to treatment, SBTI was preincubated with MCGs for 30 min before treatment. GAPDH serves as a loading control. Data are median and scatter dot plot; $n=3$. One-Way ANOVA or Kruskal-Wallis H was used to test the difference between groups (A: c-Caspase3: $F=21.113$, $P=0.000$; NOX2: $H=10.385$, $P=0.016$; NOX4: $F=64.704$, $P=0.000$; p-PKC β 1: $H=8.641$, $P=0.034$; B: c-Caspase3: $H=11.633$, $P=0.020$; NOX2: $H=10.700$, $P=0.030$; NOX4: $F=49.391$, $P=0.000$; p-PKC β 1: $F=16.048$, $P=0.000$; C: c-Caspase3: $F=19.756$, $P=0.002$; NOX2: $H=7.200$, $P=0.027$; NOX4: $H=6.489$, $P=0.039$; D: c-Caspase3: $H=7.200$, $P=0.027$; NOX2: $H=7.200$, $P=0.027$; NOX4: $F=75.410$, $P=0.000$; p-PKC β 1: $F=155.908$, $P=0.000$). * $P < 0.05$, ** $P < 0.001$.

图4 肥大细胞通过PAR2-PKC β 1-NOX2/NOX4通路加重酒精诱导的心肌细胞凋亡

Fig. 4 Mast cells exacerbate alcohol-induced cardiomyocyte apoptosis via the PAR2-PKC β 1-NOX2/NOX4 pathway

抑制剂 SBTI (soybean trypsin inhibitor) 对乳大鼠心肌细胞进行预处理能显著降低 MCGs 引起的 c-Caspase3、NOX2、NOX4 及 p-PKC β 1 表达上调 (图4D)。

3 讨论

关于酒精性心肌病的研究多集中于酒精及其代谢物的直接心脏毒性。然而,越来越多的证据表

明心肌病的发生涉及微环境中复杂的细胞间相互作用,特别是炎症细胞的浸润与激活。例如,中性粒细胞在心肌梗死后的早期修复中至关重要,但其持续活化可能加重心肌损伤并促进心力衰竭进展^[14-15];T细胞聚集亦与非缺血性心力衰竭的病理肥厚及纤维化相关^[16]。肥大细胞能够分泌各类炎症介质,在多种心血管疾病中起重要作用,例如在心肌梗死后的心脏重塑过程中,肥大细胞释放的介质能够调节免疫细胞的反应,影响心肌损伤的修复过程^[17],其在终末期心力衰竭患者心肌中的数量也显著增加^[7],但其在酒精性心肌病中的作用尚未见报道。

本研究显示,长期酒精摄入虽未改变小鼠心肌肥大细胞数量,但可激活肥大细胞,表现为脱颗粒率增加以及心肌糜蛋白酶、氧化应激标志物和凋亡水平升高;肥大细胞稳定剂色甘酸钠可部分逆转上述变化,提示肥大细胞功能状态(而非数量)在酒精性心肌病进展中更具意义。尽管有体外研究表明酒精可抑制肥大细胞活化^[18],但多项动物实验证实酒精摄入能增加外周组织肥大细胞数量并促进其活化^[19-20],这也与本研究结果相符。上述实验结果的差异,可能与体内外微环境不同,以及酒精在体内代谢产生多种活性物质有关。

本研究还发现,MCGs与酒精在诱导心肌细胞凋亡方面表现出显著的协同作用。这一发现提示,在酒精性心肌病的病理演进过程中,心肌损伤并非仅源于酒精及其代谢产物的直接细胞毒性作用,肥大细胞在酒精或其他因素刺激下脱颗粒释放的生物活性物质扮演了重要的“第二打击”角色。这些颗粒组分可能通过激活特定的受体信号通路,与酒

精诱发的氧化应激和代谢紊乱产生叠加效应,从而进一步加剧心肌细胞的凋亡进程,并加速心肌纤维化与心室重构的发生。我们接下来进一步探究这些颗粒成分通过何种途径引起心肌损伤。

PAR2是一种位于细胞膜表面的G蛋白偶联受体,能够被肥大细胞来源的蛋白酶激活^[21-22],继而触发PKC等下游信号^[23]。结果显示,抑制PAR2可减弱MCGs诱导的p-PKC β 1、NOX2、NOX4及c-Caspase3表达,提示PAR2激活参与该信号传导过程。PKC是PAR2下游的重要信号分子^[24-25],既往研究提示PKC β 1-NOX2/NOX4介导的氧化应激参与酒精引起的心肌损伤^[26]。在本研究中,抑制PKC β 1亦能降低NOX2、NOX4及凋亡相关蛋白表达,表明PAR2-PKC β 1信号通路参与介导MCGs所致心肌损伤。肥大细胞所含的多种炎症介质与心血管疾病相关,比如组胺、类胰蛋白酶、白三烯及细胞因子(如TNF- α 、IL-1、IL-6等),通过多途径参与动脉粥样硬化、心脏瓣膜病等心血管疾病的发生发展^[27]。此外,糜蛋白酶因其促进血管紧张素II生成^[9]及直接诱导心肌细胞凋亡^[5]的作用而备受关注。本研究进一步研究表明,抑制糜蛋白酶可显著减轻MCGs诱导的心肌细胞损伤,提示糜蛋白酶是肥大细胞颗粒参与酒精性心肌病进展的关键介质之一。

综上所述,本研究揭示了肥大细胞在酒精性心肌病心肌重构中的关键作用,其机制涉及肥大细胞激活后通过颗粒物质释放触发PAR2-PKC β 1-NOX2/NOX4信号轴,进而促进氧化应激损伤和心肌细胞凋亡。本研究还发现了肥大细胞颗粒中的糜蛋白酶在酒精性心肌损伤中的直接贡献,这些发现为酒精性心肌病的治疗提供了新的潜在干预靶点。

参考文献

- [1] Domínguez F, Adler E, García-Pavía P. Alcoholic cardiomyopathy: an update [J]. Eur heart J, 2024, 45 (26): 2294-2305.
- [2] Rehm J, Assanangkornchai S, Hendershot CS, et al. Alcohol use disorders [J]. Lancet (London, England), 2025, 406 (10516): 2269-2281.
- [3] 王昭,吴其明.酒精性心肌病临床特点及治疗研究进展[J].中国医学前沿杂志(电子版), 2019, 11(06): 7-11.
Wang Z, Wu QM. Progress on clinical characteristics and treatment of alcoholic cardiomyopathy [J]. Chin J Front Med Sci (Electron Ed), 2019, 11(06): 7-11.
- [4] Ihekire NL, Okobi OE, Adedoye EA, et al. Heartache in a bottle: understanding alcoholic cardiomyopathy [J]. Cureus, 2023, 15(8): e42886.
- [5] Hara M, Matsumori A, Ono K, et al. Mast cells cause apoptosis of cardiomyocytes and proliferation of other intramyocardial cells in vitro [J]. Circulation, 1999, 100(13): 1443-1449.
- [6] Ribatti D, D'Amati A. Hematopoiesis and mast cell development [J]. Int J Mol Sci, 2023, 24(13): 10679.

- [7] Patella V, Marino I, Arbustini E, et al. Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy [J]. *Circulation*, 1998, 97 (10): 971-978.
- [8] Batlle M, Perez-Villa F, Lazaro A, et al. Correlation between mast cell density and myocardial fibrosis in congestive heart failure patients [J]. *Transplant Proc*, 2007, 39 (7): 2347-2349.
- [9] Dell'Italia LJ, Collawn JF, Ferrario CM. Multifunctional role of chymase in acute and chronic tissue injury and remodeling [J]. *Circ Res*, 2018, 122(2): 319-336.
- [10] 刘丹, 宋海旭, 闫承慧, 等. E1A激活基因阻遏子基因对血管紧张素 II 刺激后小鼠原代心肌细胞凋亡影响[J]. *临床军医杂志*, 2021, 49(10): 1113-1116.
- Liu D, Song HX, Yan CH, et al. Effects of E1A activating gene repressor gene on apoptosis of primary mouse cardiomyocytes after angiotensin II stimulation [J]. *Clin J Med Officers*, 2021, 49(10): 1113-1116.
- [11] Hu Y, Zabini D, Gu W, et al. The role of the human immune system in chronic hypoxic pulmonary hypertension [J]. *Am J Respirat Crit Care Med*, 2018, 198(4): 528-531.
- [12] Jensen BM, Swindle EJ, Iwaki S, et al. Generation, isolation, and maintenance of rodent mast cells and mast cell lines [J]. *Curr Protoc Immunol*, 2006, 3: 3.23.1-3.23.13. doi: 10.1002/0471142735.im0323s74.
- [13] He JG, Chen SL, Huang YY, et al. The nonpeptide AVE0991 attenuates myocardial hypertrophy as induced by angiotensin II through downregulation of transforming growth factor- β 1/Smad2 expression [J]. *Heart Vessels*, 2010, 25 (5): 438-443.
- [14] Horckmans M, Ring L, Duchene J, et al. Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype [J]. *Eur Heart J*, 2017, 38(3): 187-197.
- [15] Antipenko S, Mayfield N, Jinno M, et al. Neutrophils are indispensable for adverse cardiac remodeling in heart failure [J]. *J Mol Cell Cardiol*, 2024, 189: 1-11.
- [16] Nevers T, Salvador AM, Grodecki-Pena A, et al. Left ventricular T-cell recruitment contributes to the pathogenesis of heart failure [J]. *Circ Heart Fail*, 2015, 8(4): 776-787.
- [17] Kologrivova I, Shtatolkina M, Suslova T, et al. Cells of the immune system in cardiac remodeling: main players in resolution of inflammation and repair after myocardial infarction [J]. *Front Immunol*, 2021, 12: 664457.
- [18] Toivari M, Maki T, Suutarla S, et al. Ethanol inhibits IgE-induced degranulation and cytokine production in cultured mouse and human mast cells [J]. *Life Sci*, 2000, 67 (23): 2795-2806.
- [19] Wimberly AL, Forsyth CB, Khan MW, et al. Ethanol-induced mast cell-mediated inflammation leads to increased susceptibility of intestinal tumorigenesis in the APC Delta468 min mouse model of colon cancer [J]. *Alcohol Clin Exp Res*, 2013, 37(1): E199-E208.
- [20] Mendes LO, Amorim JP, Teixeira GR, et al. Mast cells and ethanol consumption: interactions in the prostate, epididymis and testis of UChB rats [J]. *Am J Reprod Immunol*, 2011, 66 (3): 170-178.
- [21] Sharma R, Prasad V, McCarthy ET, et al. Chymase increases glomerular albumin permeability via protease-activated receptor-2 [J]. *Mol Cell Biochem*, 2007, 297 (1-2): 161-169.
- [22] Groschwitz KR, Wu D, Osterfeld H, et al. Chymase-mediated intestinal epithelial permeability is regulated by a protease-activating receptor/matrix metalloproteinase-2-dependent mechanism [J]. *Am J Physiol Gastrointest Liver Physiol*, 2013, 304(5): G479-G489.
- [23] Steinhoff M, Buddenkotte J, Shpacovitch V, et al. Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response [J]. *Endocr Rev*, 2005, 26(1): 1-43.
- [24] van der Merwe JQ, Moreau F, Macnaughton WK. Protease-activated receptor-2 stimulates intestinal epithelial chloride transport through activation of PLC and selective PKC isoforms [J]. *Am J Physiol Gastrointest Liver Physiol*, 2009, 296(6): G1258-G1266.
- [25] Park GH, Jeon SJ, Ko HM, et al. Activation of microglial cells via protease-activated receptor 2 mediates neuronal cell death in cultured rat primary neuron [J]. *Nitric Oxide*, 2010, 22(1): 18-29.
- [26] Tan Y, Li X, Prabhu SD, et al. Angiotensin II plays a critical role in alcohol-induced cardiac nitrative damage, cell death, remodeling, and cardiomyopathy in a protein kinase C/nicotinamide adenine dinucleotide phosphate oxidase-dependent manner [J]. *J Am Coll Cardiol*, 2012, 59(16): 1477-1486.
- [27] 杜燕欢, 张世忠. 肥大细胞与心血管疾病关系研究进展 [J]. *中国细胞生物学报*, 2023, 45(11): 1705-1714.
- Du YH, Zhang SZ. Research progress on the relationship between mast cells and cardiovascular disease [J]. *Chin J Cell Biol*, 2023, 45(11): 1705-1714.