·基础研究·

钠-葡萄糖共转运体2抑制剂通过调节巨噬细胞极化和旁分泌改善心肌纤维化

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【摘 要】 目的 验证钠-葡萄糖共转运体2抑制剂(SGLT2i)能否在缺氧/复氧(H/R)条件下通过改变巨噬细胞旁分泌来调节心脏成纤维细胞(CFs)的激活,从而改善心肌纤维化。**方法** 将27只C57BL/6雄性小鼠按随机数表法随机分为3组,每组9只:(1)假手术组(sham组);(2)缺血再灌注损伤(IRI)组;(3)缺血再灌注损伤+SGLT2i组(IRI+SGLT2i组)。28 d后采用超声心动图和免疫荧光染色检测心肌纤维化和不良重构。培养RAW264.7细胞,分为以下3组:(1)阴性对照(NC组);(2)缺氧/复氧组(H/R组);(3)缺氧/复氧+SGLT2i组(H/R+SGLT2i组)。采用免疫荧光染色、Western blotting(WB)检测M2极化,使用酶联免疫吸附试验(ELISA)检测转化生长因子β(TGF-β)、白细胞介素1β(IL-1β)、肿瘤坏死因子α(TNF-α)、白细胞介素6(IL-6)、血小板衍生生长因子a(PDGF-a)的分泌情况。提取小鼠CFs分为以下3组:(1)阴性对照(NC组);(2)缺氧/复氧组(H/R组);(3)缺氧/复氧+SGLT2i组(H/R+SGLT2i组)。在各组中加入相应的巨噬细胞上清液,检测CFs的激活情况。采用GraphPad Prism 8软件进行数据分析。多组间比较采用单因素方差分析,2组间比较采用Tukey's事后检验。**结果** 动物模型术后28 d超声心动图结果显示:与IRI组相比,IRI+SGLT2i组表现出较高的左室射血分数和左室缩短分数,而左室收缩末期容积、左室舒张末期容积、左室收缩期内径和左室舒张期内径都较小(均$P<0.05$)。免疫荧光染色结果显示:与IRI组相比,IRI+SGLT2i组的Ⅰ型和Ⅲ型胶原蛋白含量降低($P<0.05$),且天狼星红染色结果显示Ⅰ型和Ⅲ型胶原蛋白比例明显下降($P<0.05$)。细胞模型WB结果显示:与H/R组相比,H/R+SGLT2i组的M2标志物精氨酸酶1(Arg1)含量较多,M1标志物诱导型一氧化氮合酶(iNOS)含量较少($P<0.05$)。ELISA实验证实:TGF-β、IL-1β、TNF-α、IL-6及PDGF-a在H/R+SGLT2i组的含量相对H/R组更少($P<0.05$)。WB结果证实:H/R+SGLT2i组CFs表达的α-平滑肌肌动蛋白和胶原蛋白的表达量低于H/R组($P<0.05$)。**结论** SGLT2i可改善IRI诱导的心肌纤维化。SGLT2i抑制M1极化,促进M2极化,抑制炎症和纤维化相关细胞因子的分泌。SGLT2i处理RAW264.7细胞上清液可抑制CFs激活,证实SGLT2i通过调节巨噬细胞的旁分泌来抑制CFs的激活,改善心室不良重构。

【关键词】 心肌纤维化;巨噬细胞极化;钠-葡萄糖共转运体2抑制剂;缺血再灌注损伤;缺氧复氧;旁分泌

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Sodium-glucose cotransporter 2 inhibitor attenuates cardiac fibrosis by altering macrophage polarization and regulating its paracrine

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【Abstract】 Objective To verify whether sodium-glucose cotransporter 2 inhibitor (SGLT2i) can regulate the activation of cardiac fibroblasts (CFs) by changing macrophage paracrine under hypoxic/reoxygenation (H/R) condition, and thus attenuate myocardial fibrosis. **Methods** A total of 27 C57BL/6 male mice were randomly divided into sham group, ischemia-reperfusion injury (IRI) group and IRI+SGLT2i group, with 9 animals in each groups. In 28 d later, echocardiography and immunofluorescence assay were used to detect myocardial fibrosis and adverse remodeling. RAW264.7 cells were assigned into negative control (NC) group, H/R group and H/R+SGLT2i group. M2 polarization were observed with immunofluorescence assay and Western blotting. Secretion of transforming growth factor beta (TGF-β), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin 6(IL-6), and platelet-

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derived growth factor-a (PDGF-a) were measured with enzyme linked immunosorbent assay (ELISA). Primarily cultured mouse CFs were divided into NC group, H/R group and H/R+SGLT2i group. Then macrophage supernatant was added into the culture medium of the CFs to observe the cell activation. GraphPad Prism 8 statistics was used for statistical analysis. Multiple group comparisons were conducted using one-way analysis of variance, while data comparison between two groups was conducted using Tukey′ post hoc test. **Results** *In vivo*, echocardiographic findings at 28 d postoperatively showed that the IRI+SGLT2i group exhibited higher left ventricular ejection fraction and left ventricular shortening fraction, whereas lower left ventricular end-systolic volume, left ventricular end-diastolic volume, left ventricular internal systolic diameters, and left ventricular internal diastolic diameters when compared with the IRI group (all $P<0.05$). Immunofluorescence assay indicated that the IRI+SGLT2i group had reduced contents of type Ⅰ and type Ⅲ collagen, and Sirius red staining showed a decreased ratio of type Ⅰ to type Ⅲ collagen compared with the IRI group ($P<0.05$). *In vitro*, Western blotting showed that the H/R+SGLT2i group had increased ratio of M2 marker, Arg1, and less ratio of M1 marker, iNOS, compared to the H/R group ($P<0.05$). ELISA indicated less abundant TGF-β, IL-1β, TNF-α, IL-6 and PDGF-a, were observed in the H/R+SGLT2i group than the H/R group ($P<0.05$). Western blotting results confirmed that the expression of α-SMA and collagen in the CFs in the H/R+SGLT2i group was lower than that in the H/R group ($P<0.05$). **Conclusion** SGLT2i ameliorates IRI-induced myocardial fibrosis. It can inhibit M1 polarization, promote M2 polarization, and suppress the secretion of inflammatory and fibrosis-related cytokines. Its treatment for RAW264.7 cell supernatant can inhibit the activation of CFs. Our findings confirm that SGLT2i suppresses the activation of CFs and improves poor ventricular remodeling by regulating the paracrine secretion of macrophages.

【Key words】 myocardial fibrosis; macrophage polarization; sodium-glucose cotransporter 2 inhibitor; ischemia-reperfusion injury; hypoxia and reoxygenation; paracrine

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心血管疾病是全世界死亡和发病的主要原因，造成了巨大的社会经济负担[1]。急性心肌梗死(acute myocardial infarction，AMI)后早期干预，特别是及时再灌注治疗，大大改善了生存率。即便如此，急性心肌梗死后的左心室重塑和随后的心力衰竭(heart failure，HF)仍然是一个严峻的临床挑战[2]。在心肌梗死后的修复过程中，成纤维细胞表达 α-平滑肌肌动蛋白(alpha-smooth muscle actin，α-SMA)被激活成为肌成纤维细胞，这是心肌纤维化的一个关键步骤。肌成纤维细胞合成并分泌大量细胞外基质，在心肌纤维化的发生和发展中发挥重要作用[3]。心肌梗死后，巨噬细胞被招募到心脏，作为关键的炎症调节细胞发挥重要功能。既往研究表明，巨噬细胞和心脏成纤维细胞(cardiac fibroblasts，CFs)之间的相互作用促进心脏重塑[4]。心肌梗死期间 M1 到 M2 巨噬细胞表型的延迟转换被认为是不良心室重塑(adverse ventricular remodeling，AVR)的主要因素之一。钠-葡萄糖共转运体 2 抑制剂(sodium-glucose cotransporter 2 inhibitor，SGLT2i)是一种用于治疗糖尿病的降糖药物[5]，在大鼠心肌梗死模型中 SGLT2i 可以通过调节巨噬细胞极化来缓解纤维化[3]。然而，SGLT2i 如何在再灌注治疗后抑制纤维化及巨噬细胞旁分泌在其中的作用仍有待研究。

1 材料与方法

1.1 实验动物与分组

选用购自北京斯贝福生物有限公司[SCXK(Beijing) 2019-0010] C57BL/6 小鼠(雄性，8 周龄，18~22 g，$n=27$)，构建小鼠心肌缺血再灌注损伤(ischemia-reperfusion injury，IRI)模型。按照随机数表法随机将小鼠分为 Sham 组、IRI 组和 IRI+SGLT2i 组，每组 9 只。将 IRI 组和 IRI+SGLT2i 组小鼠吸入异氟醚麻醉，暴露心脏，在左耳廓下缘 2 mm 处结扎前降支，快速缝合胸腔，90 min 后解开[6]。Sham 组小鼠采用完全相同的方法，但不结扎前降支[7]。IRI+SGLT2i 组术后给予含达格列净的饮用水，1 mg/(kg·d)[8]。本实验经中国人民解放军总医院动物实验伦理委员会批准(2022-X18-96)。

1.2 超声心动图

在术后 28 d 对小鼠进行经胸超声心动图(Vevo2100 成像系统，Visual sonic，多伦多，加拿大)检测，小鼠体温维持在 36.9℃~37.3℃，心率维持在 400~550 次/min。使用 M 型超声心动图记录至少 6 个心动周期，计算左室射血分数(left ventricular ejection fraction，LVEF)、左室舒张期内径(left ventricular internal diastolic diameters，LVIDd)、左室收缩末期容积(left ventricular end-systolic volume，LVESV)、左室舒张末期容积(left ventricular end-diastolic volume，LVEDV)、左室缩短分数(left ventricular shortening fraction，LVFS)和左室收缩期内径(left ventricular internal systolic diameters，LVIDs)。

1.3 免疫组织化学染色和免疫荧光染色

术后 28 d 处死小鼠，采用 4%多聚甲醛固定心脏并切片，天狼星红染色和免疫荧光染色检测纤维化。用Ⅰ型胶原蛋白(collagen Ⅰ，Col Ⅰ；Servicebio，GB110223，1∶200)和Ⅲ型胶原蛋白(collagen Ⅲ，ColⅢ；Servicebio，GB111629，1∶200)标记纤维化。二抗为 Cy3-山羊抗小鼠 IgG(Servicebio，GB21303，1∶300)。

1.4 RAW264.7细胞培养和H/R模型建立

RAW264.7细胞(Procell Life Science and Technology, CL-0190)在含有DMEM(Gibco,11965092)、10%胎牛血清、1% L-谷氨酰胺和1%青霉素-链霉素的完全培养基培养。将RAW264.7分为以下3组:NC组的RAW264.7细胞在37℃、5% CO_2条件下培养;缺氧/复氧(hypoxic/reoxygenation, H/R)组的RAW264.7在94% N_2、5% CO_2、1% O_2的培养箱缺氧培养12 h,然后在37℃、5% CO_2下复氧6 h;H/R+SGLT2i组在H/R开始时用SGLT2i(10 μmol/L)处理RAW264.7细胞[9]。

1.5 小鼠CFs原代培养及H/R模型的建立

用眼科剪取小鼠心室,用含0.08%胰蛋白酶(Gibco, 25200056)和0.05% Ⅱ型胶原酶(Sigma, 1148090)的磷酸盐缓冲液(phosphate buffer solution, PBS)消化5 min,用含10%胎牛血清的完全培养基终止消化。吸出上清液,通过添加消化液继续消化剩余的组织碎片,直到组织碎片消失。随后培养60 min,更换培养基以纯化CFs。提取成功的原代成纤维细胞分为以下3组:NC组的CFs在37℃、5% CO_2条件下的完全培养基中培养;H/R组在培养基中加入H/R组巨噬细胞培养基上清液后,CFs在94% N_2、5% CO_2、1% O_2的培养箱缺氧培养12 h,然后在37℃、5% CO_2下复氧36 h;H/R+SGLT2i组在培养基中加入H/R+SGLT2i组巨噬细胞培养基上清液后,CFs在94% N_2、5% CO_2、1% O_2的培养箱缺氧培养12 h,然后在37℃、5% CO_2下复氧36 h。

1.6 免疫荧光染色

通过巨噬细胞标记物CD86和Arg1进行巨噬细胞亚型鉴定,通过成纤维细胞α-SMA和ColⅠ进行成纤维细胞激活鉴定。细胞在室温下用4%多聚甲醛溶液固定30 min,然后用0.5% Triton-100渗透细胞30 min,用1%牛血清白蛋白(bovine serum albumin,BSA)在室温下封闭2 h,用抗CD86(Proteintech, 13395-1-AP, 1∶100)、Arg1(Proteintech, 16001-1-AP, 1∶100)、α-SMA(Cell signaling, 19245s, 1∶200)、ColⅠ(Proteintech, 14695-1-AP, 1∶100)的一抗在4℃下孵育过夜。PBS清洗细胞,荧光二抗(华兴博创, HX2027)室温处理1 h。用4,6-二氨基-2-苯基吲哚(4′,6-diamidino-2-phenylindole, DAPI)染色。从每个样本中随机选择三个区域,用荧光显微镜观察。

1.7 Western blotting

用放射免疫沉淀法裂解缓冲液(radio immunoprecipitation assay, RIPA;Solarbio, R0020)将细胞在冰上裂解30 min。在4℃下,以14 000 转/min的转速离心30 min,以清除死细胞碎片。采用二喹啉甲酸法蛋白测定试剂盒(Solarbio, PC0020)检测蛋白浓度。样品经十二烷基硫酸钠聚丙烯酰胺凝胶电泳后转移到0.22 μm聚偏二氟乙烯膜上。将细胞膜在5%的牛奶中封闭1 h,在4℃下孵育一抗过夜,室温下与抗兔二抗在摇床上孵育1 h。用Image J软件分析蛋白的相对表达量。使用以下抗体:CD86(Proteintech, 13395-1-AP, 1∶100),Arg1(Proteintech, 16001-1-AP, 1∶100),诱导型一氧化氮合酶(inducible nitric oxide synthase,iNOS; Abcam, ab213480, 1∶100),甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, GAPDH; Proteintech, 60004-1-Ig, 1∶50),α-SMA(Cell signaling, 19245s, 1∶200),ColⅠ(Proteintech, 14695-1-AP, 1∶100)。

1.8 酶联免疫吸附试验

收集巨噬细胞上清液,采用酶联免疫吸附试验(enzyme linked immunosorbent assay,ELISA)试剂盒(Proteintech)检测白细胞介素1β(interleukin-1 beta, IL-1β)、肿瘤坏死因子α(tumor necrosis factor alpha, TNF-α)、白细胞介素6(interleukin 6,IL-6)、转化生长因子β(transforming growth factor beta,TGF-β)和血小板衍生生长因子a(platelet-derived growth factor a, PDGF-a)含量。操作均严格按照说明书进行。

1.9 统计学处理

采用GraphPad Prism 8统计软件进行数据分析。所有数据至少独立重复3次。符合正态分布的计量资料用均数±标准差($\bar{x}\pm s$)表示,多组间比较采用单因素方差分析检验,2组间比较采用Tukey's事后检验。$P<0.05$为差异有统计学意义。

2 结 果

2.1 模型建立28 d后心功能指标的比较

使用小型动物超声系统测量了Sham、IRI和IRI+SGLT2i组的LVEF、LVEDV、LVESV、LVIDd、LVIDs和FS。结果显示,小鼠模型建立后28 d,与IRI组相比,IRI+SGLT2i组的LVEF及FS更高($P<0.05$);与IRI组相比,LVIDd、LVEDV、LVID及LVESV在IRI+SGLT2i组更低(均$P<0.05$;图1)。

2.2 模型建立28 d后心脏纤维化程度的比较

在术后28 d处死小鼠获得心脏后,用苏木红染色法评估纤维化。普通光镜结果显示,IRI+SGLT2i组的纤维化面积小于IRI组;偏光显微镜结果显示,IRI+SGLT2i组的红绿面积比小于IRI组(均$P<0.05$)。用组织免疫荧光染色法观察SGLT2i对ColⅠ和ColⅢ的影响,结果显示ColⅠ的累积光密度(integrated optical density,IOD)和ColⅢ的IOD在IRI+SGLT2i组中都比IRI组少(均$P<0.05$;图2)。

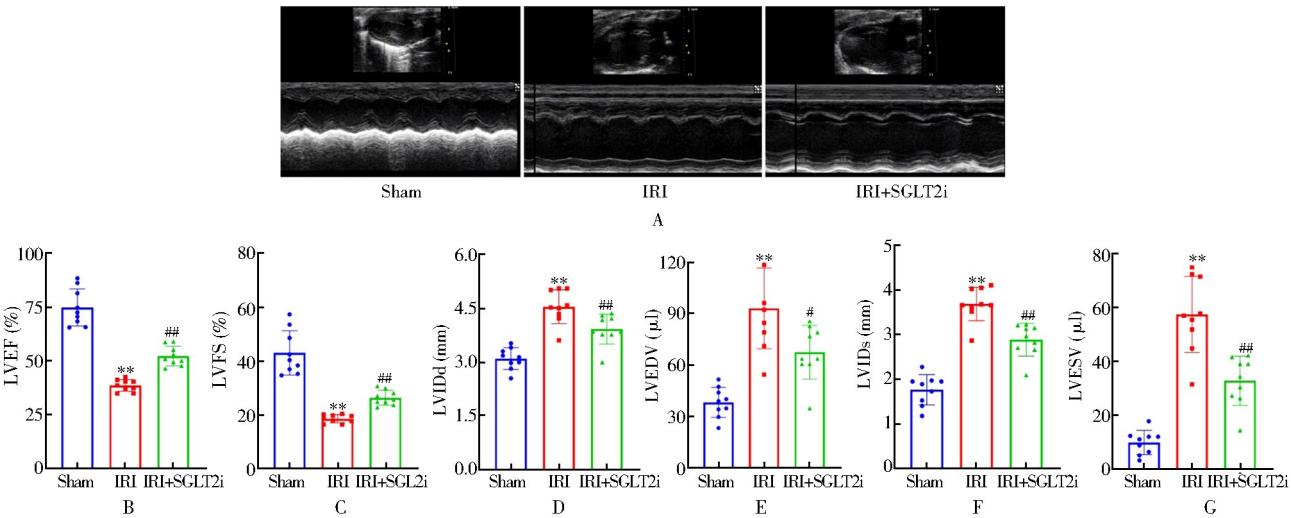


图 1 模型建立 28 d 后 LVEF,LVFS,LVIDd,LVEDV,LVIDs,LVESV 的比较

Figure 1 Comparison of LVEF, LVFS, LVIDd, LVEDV, LVIDs, LVESV 28 d after model establishment ($n=9$)

A: echocardiograms; B: LVEF; C: LVFS; D: LVIDd,; E: LVEDV; F: LVIDs; G: LVESV. SGLT2i: sodium-glucose cotransporter 2 inhibitor; AVR: adverse ventricular remodeling; IRI: ischemia-reperfusion injury; LVEF: left ventricular ejection fraction; LVFS: left ventricular shortening fraction; LVIDd: left ventricular internal diastolic diameters; LVEDV: left ventricular end-diastolic volume; LVIDs: left ventricular internal systolic diameters; LVESV: left ventricular end-systolic volume. Compared with sham group, $^{**}P<0.01$; compared with IRI group, $^{\#}P<0.05$, $^{\#\#}P<0.01$.

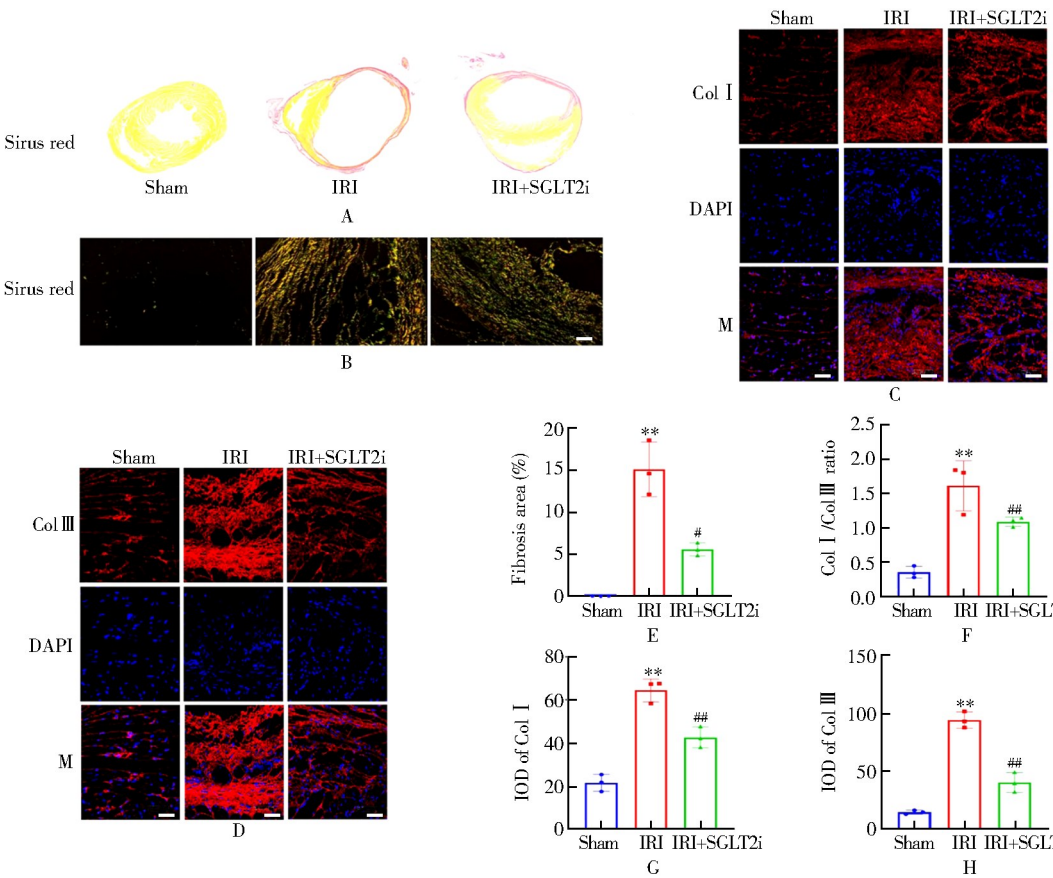


图 2 模型建立 28 d 后,纤维化面积,胶原蛋白含量及比例的比较

Figure 2 Comparison of fibrosis area, collagen content and proportion 28 d after model establishment ($n=3$)

A: sirius red staining, ordinary light microscopy (scale bar=2 000 μm); B: sirius red staining, polarized light microscopy (scale bar=50 μm); C: scale bar=50 μm; D: scale bar=50 μm; E: quantification of fibrosis area; F: quantification of ratio of Col Ⅰ to Col Ⅲ; G: quantification of IOD of Col Ⅰ; H: quantification of IOD of Col Ⅲ. SGLT2i: sodium-glucose cotransporter 2 inhibitor; AVR: adverse ventricular remodeling; IRI: ischemia-reperfusion injury; Col Ⅰ: collagen Ⅰ; DAPI: 4′, 6-diamidino-2-phenylindole; M: merged; Col Ⅲ: collagen Ⅲ; IOD: integrated optical density. Compared with sham group, $^{**}P<0.01$; compared with IRI group, $^{\#}P<0.05$, $^{\#\#}P<0.01$.

2.3 H/R 条件下 SGLT2i 对巨噬细胞极化的影响

为了研究 H/R 对巨噬细胞极化的影响以及 SGLT2i 的调节作用,本研究构建了巨噬细胞 H/R 模型。用蛋白质免疫印迹(Western blot, WB)检测 M1 巨噬细胞的标志物(CD86 和 iNOS)以及 M2 巨噬细胞的标志物(Arg1)。本研究发现 SGLT2i 抑制了巨噬细胞向 M1 的极化,并促进其在 H/R 时向 M2 的极化。WB 显示,与 NC 组相比,H/R 组的巨噬细胞表达 Arg1、iNOS 及 CD86 的水平更高;与 H/R 组相比,H/R+SGLT2i 组的巨噬细胞 Arg1 表达水平增加,iNOS 及 CD86 表达水平下降(均 $P<0.05$)。免疫荧光结果表明,与 NC 组相比,H/R 刺激的 RAW264.7 Arg1 及 CD86 表达水平升高;H/R+SGLT2i 组比 H/R 组的 Arg1 表达水平更高,CD86 表达水平更低(均 $P<0.05$)。流式细胞实验证实,H/R 组的 F4/80 和 CD206 与 F4/80 和 CD86 阳性细胞比例均高于 NC 组;H/R+SGLT2i 组的 F4/80 和 CD86 阳性细胞比例低于 H/R 组,F4/80 和 CD206 阳性细胞比例高于 H/R 组($P<0.05$;图 3)。

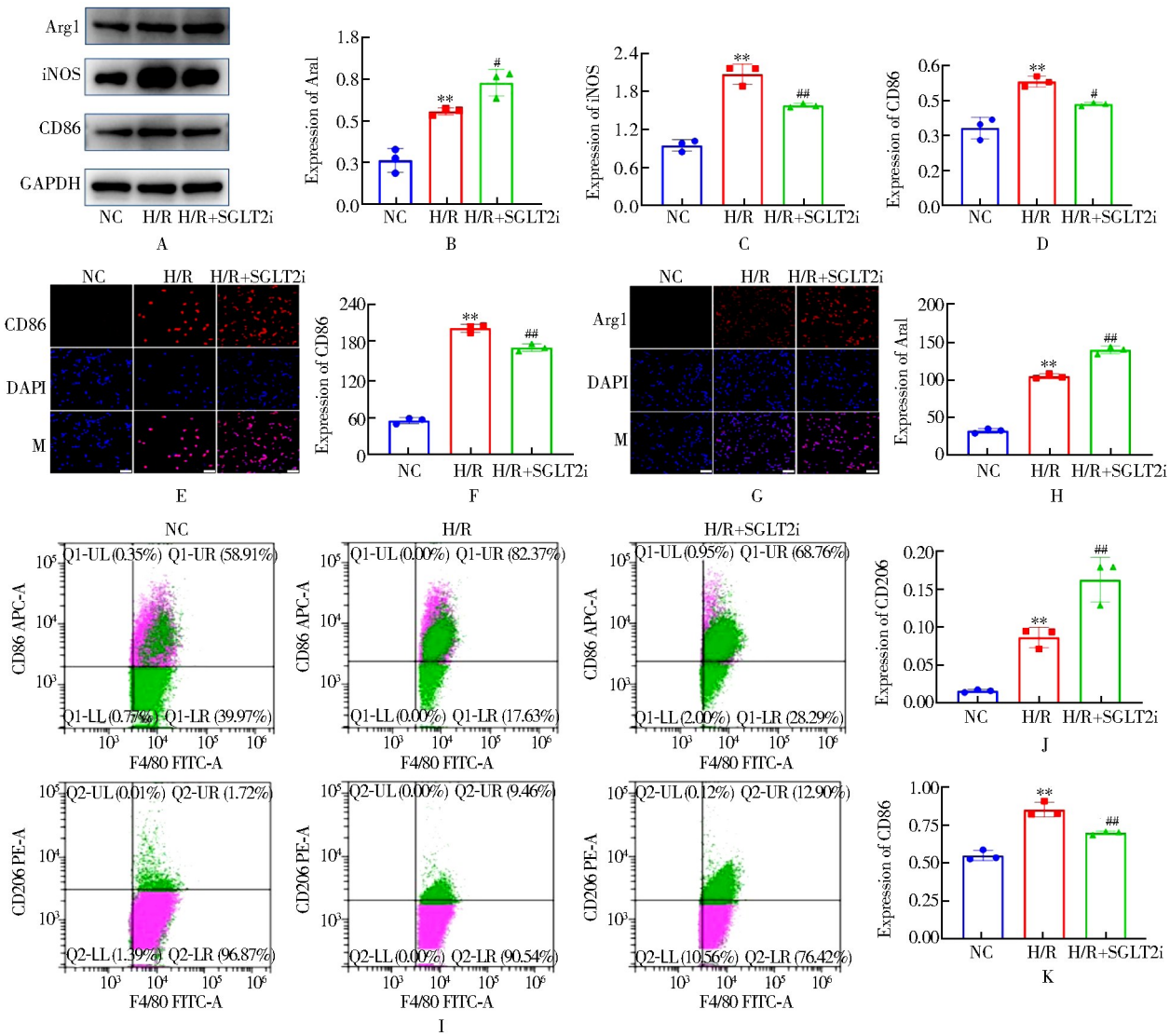


图 3 NC 组,H/R 组和 H/R+SGLT2i 组巨噬细胞极化程度的比较

Figure 3 Comparison of macrophage polarization in NC, H/R and H/R+SGLT2i groups ($n=3$)

A: expression of iNOS, CD86 and Arg1 deceted by WB; B: quantitative analysis of Arg1 expression; C: quantitative analysis of iNOS expression; D: quantitative analysis of CD86 expression; E: immunofluorescence staining (scale bar=80 μm); F: quantitative analysis of cellular IOD of CD86; G: immunofluorescence staining (scale bar=80 μm); H: quantitative analysis of cellular IOD of Arg1; I: flow cytometry; J: quantitative analysis of CD86-positive cells; K: quantitative analysis of CD206-positive cells. Compared with sham group, $^{**}P<0.01$; compared with IRI group, $^{\#}P<0.05$, $^{\#\#}P<0.01$. SGLT2i: sodium-glucose cotransporter 2 inhibitor; AVR: adverse ventricular remodeling; IRI: ischemia-reperfusion injury; M: merged; H/R: hypoxia/reoxygenation; NC: negative control. iNOS: inducible nitric oxide synthase; WB: Western blotting; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

2.4 H/R 条件下 SGLT2i 对巨噬细胞分泌促炎和促纤维化细胞因子的影响

收集各组巨噬细胞的上清液，并通过 ELISA 测定其细胞因子含量。与 NC 组相比，H/R 处理增加了 PDGF-a、TGF-β、TNF-α、IL-6 和 IL-1β 含量；而 PDGF-a、TGF-β、TNF-α、IL-6 和 IL-1β 含量在 H/R+SGLT2i 组下降（均 $P<0.05$；图 4）。

2.5 SGLT2i 处理的巨噬细胞上清液对成纤维细胞活化的影响

在向成纤维细胞培养基中添加巨噬细胞上清液和 H/R 处理后，测量成纤维细胞的激活程度。激活后的成纤维细胞转变为肌成纤维细胞，高表达 α-SMA 和 Col Ⅰ。WB 实验证实，与 NC 组相比，H/R 组成纤维细胞表达更高的 α-SMA 和 Col Ⅰ；H/R+SGLT2i 组成纤维细胞表达的 α-SMA 和 Col Ⅰ比 H/R 组低（均 $P<0.05$）。免疫荧光结果显示，与 NC 组相比，H/R 组的成纤维细胞表达更高的 α-SMA 和 Col Ⅰ；H/R+SGLT2i 组成纤维细胞表达的 α-SMA 和 Col Ⅰ比 H/R 组低（均 $P<0.05$）。这些数据表明，SGLT2i 通过调节巨噬细胞的旁分泌来抑制 H/R 处理的成纤维细胞激活（图 5）。

3 讨 论

在动物实验中，我们验证了 SGLT2i 抑制缺血再灌注后的心肌纤维化，降低了胶原蛋白含量，降低了Ⅰ型与Ⅲ型胶原蛋白的比例，表明 SGLT2i 对心肌纤维化的抑制作用。体外实验发现 SGLT2i 促进巨噬细胞向 M2 极化，从而抑制巨噬细胞分泌促炎和促纤维化细胞因子（PDGF-a，TGF-β，IL-1β，TNF-α，IL-6）。因此，SGLT2i 发挥了抗纤维化作用。

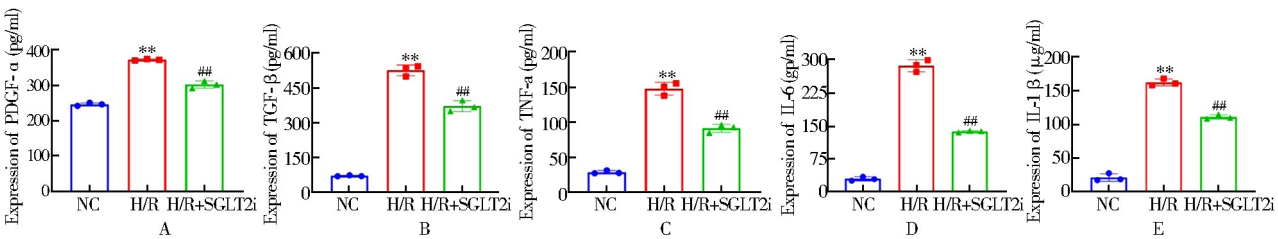


图 4 RAW264.7 上清液中 PDGF-a，TGF-β，IL-1β，TNF-α 和 IL-6 含量的比较

Figure 4 Comparison of PDGF-a, TGF-β, IL-1β, TNF-α and IL-6 in RAW264.7 supernatants ($n=3$)

A: quantitative analysis of PDGF-a; B: quantitative analysis of TGF-β; C: quantitative analysis of IL-1β; D: quantitative analysis of TNF-α; E: quantitative analysis of IL-6. SGLT2i: sodium-glucose cotransporter 2 inhibitor; H/R: hypoxia/reoxygenation; NC: negative control; PDGF-a: platelet-derived growth factor a; TGF-β: transforming growth factor beta; TNF-α: tumor necrosis factor alpha; IL-6: interleukin 6; IL-1β: interleukin-1 beta; ELISA: enzyme linked immunosorbent assay. Compared with sham group, $^{**}P<0.01$; compared with IRI group, $^{\#\#}P<0.01$.

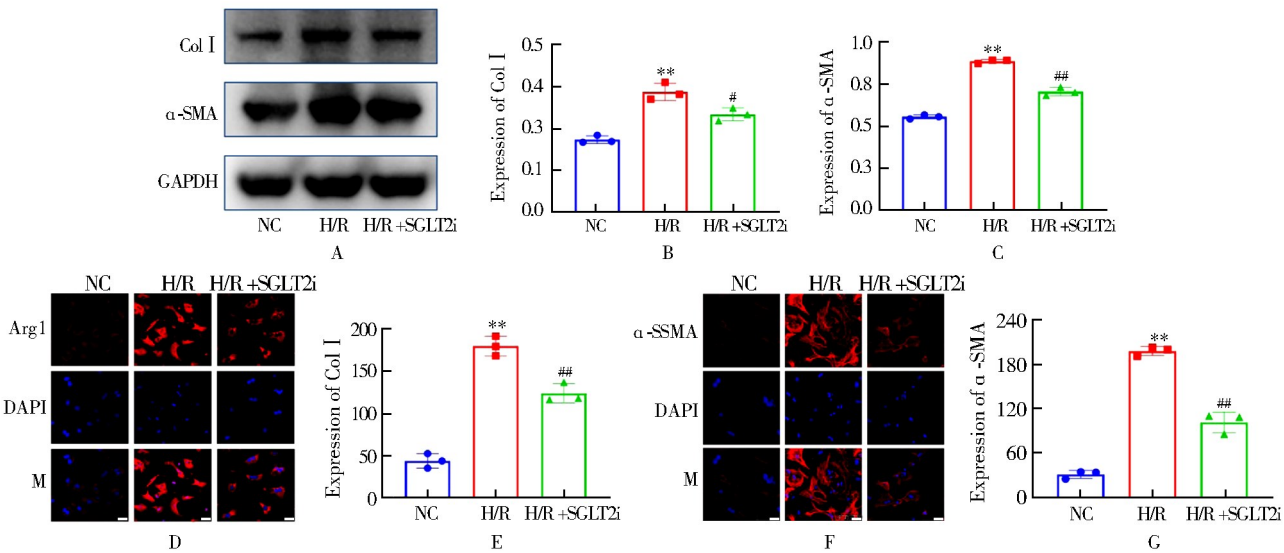


图 5 CFs 中 α-SMA 和 Col Ⅰ表达量的比较

Figure 5 Comparison of α-SMA and Col Ⅰ expressions in CFs ($n=3$)

A: expression of α-SMA and Col Ⅰ deceted by WB; B: quantitative analysis of α-SMA expression; C: quantitative analysis of Col Ⅰ expression; D: immunofluorescence staining (scale bar=80 μm); E: quantitative analysis of IOD of Col Ⅰ; F: immunofluorescence staining (scale bar=80 μm); G: quantitative analysis of the IOD of α-SMA. SGLT2i: sodium-glucose cotransporter 2 inhibitor; H/R: hypoxia/reoxygenation; NC: negative control; SGLT2i: sodium-glucose cotransporter 2 inhibitor; AVR: adverse ventricular remodeling; IRI: ischemia-reperfusion injury; Col Ⅰ: collagen Ⅰ; DAPI: 4′, 6-diamidino-2-phenylindole; M: merged; Col Ⅲ: collagen Ⅲ. CFs: cardiac fibroblasts; α-SMA: alpha-smooth muscle actin; WB: Western blotting; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. Compared with sham group, $^{**}P<0.01$; compared with IRI group, $^{\#}P<0.05$, $^{\#\#}P<0.01$.

在EMPA-REG/EMPEROR-REDUCTION/DAPA-HF试验等临床试验中,无论心力衰竭患者是否患有糖尿病,SGLT2i均降低了他们心血管死亡或住院的风险[10-12]。SGLT2i发挥心脏保护作用的机制包括降低前负荷和后负荷,改善心脏代谢和生物能量,抑制心肌Na^+/H^+交换,减少坏死和心脏纤维化,以及改变脂肪因子和心外膜脂肪组织含量[13]。然而,SGLT2i调节心脏纤维化的主要机制仍有待研究。SGLT2i减少纤维化可能与其抑制炎症反应、直接抑制CFs活化以及通过巨噬细胞极化间接调节CFs活化和抑制内皮-间充质转化有关[14]。SGLT2i对于CFs的活化增殖和迁移具有直接的抑制作用,既往研究证实SGLT2i可以抑制高糖、TGF-β或血管紧张素Ⅱ诱导的CFs活化及胶原蛋白的分泌。但对于其通过影响巨噬细胞旁分泌调节CFs活化与胶原蛋白分泌的作用尚不清楚,所以我们研究不同条件处理的巨噬细胞其上清液中促炎及促纤维化细胞因子的改变,并进一步探讨上清液中这些因子的改变对于CFs活化和胶原蛋白分泌的影响。

既往研究已经提出了成纤维细胞和巨噬细胞之间的相互作用[11]。巨噬细胞是成纤维细胞存活所需的PDGF肽的重要来源。活化的巨噬细胞在体外促进成纤维细胞的迁移和增殖,在PDGF-a阻断抗体存在时,这种迁移和增殖会减弱[12]。在成纤维细胞中,TGF-β将成纤维细胞活化为肌成纤维细胞[4]。TGF-β可以通过多种细胞类型表达,其中包括活化的巨噬细胞[4]。不同亚型的巨噬细胞分泌不同的细胞因子,在纤维化中起不同的作用。以往研究已经证实M2b可以发挥抗纤维化作用[15]。M2b移植致心肌梗死区改善大鼠心肌纤维化的研究证实:M2b减小了梗死心肌的纤维化面积,这可能与梗死组织中TGF-β、PDGF-a、血小板衍生生长因子受体(platelet-derived growth factor receptor,PDGFR)的基因表达量降低有关,并且M2b与CFs共培养可以降低CFs中PDGFR的基因表达水平[16]。然而,SGLT2i对这些因子分泌的影响尚不清楚。本实验得出结论,SGLT2i可以通过巨噬细胞极化,从而调节纤维化,与以前的研究一致[4]。我们探索了SGLT2i调节巨噬细胞极化后巨噬细胞分泌细胞因子改变的研究,并将巨噬细胞上清液加入成纤维细胞培养基,证实了SGLT2i通过改变巨噬细胞旁分泌发挥其抗纤维化作用。我们在IRI模型中验证了SGLT2i对心肌纤维化的影响,还发现SGLT2i不仅降低了胶原蛋白含量,而且还改变了Ⅰ型与Ⅲ型胶原蛋白的比例,进一步解释了SGLT2i对IRI后心肌纤维化的保护作用。

综上,本研究观察了SGLT2i通过调节巨噬细胞旁分泌改善心肌纤维化的作用,但对其具体分子机制缺乏一定的研究,后续我们将对其具体分子机制进行进一步实验研究。在此次实验分组中没有达格列净抑制组,将在后续实验中进一步完善。本实验表明SGLT2i可以通过促进M2极化抑制促纤维化和促炎细胞因子的分泌,从而发挥抑制心肌纤维化的作用。为SGLT2i改善心肌缺血再灌注损伤后AVR的作用提供了新的认识和可能的机制。

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