低氧/厌氧产品案例——糖氧剥夺（OGD）模型

文章题目：Dimethyl fumarate protects cardiomyocytes against oxygen-glucose deprivation/reperfusion (OGD/R)-induced inflammatory response and damages via inhibition of Egr-1

富马酸乙甲酯通过抑制 Egr-1 保护心肌细胞免受糖氧剥夺/再灌注(OGD/R)诱导的炎症反应和心肌损伤

文章出处： International immunopharmacology vol. 86 (2020): 106733.

郑州大学人民医院，河南省人民医院，阜外中心心血管医院

使用气体浓度：低氧（1% O_2 , 5% CO_2 , 94% N_2）；常氧（21% O_2, 5% CO_2）

主要内容：急性心肌梗死(AMI)具有较高的发病率和死亡率，动脉粥样硬化是 AMI 的主要原因之一。动脉粥样硬化斑块的破裂或侵蚀可阻碍冠状动脉硬化，从而导致缺血性损伤和心肌细胞凋亡的急性炎症反应。富马酸二甲酯（DMF）是一种富马酸二酯，用于治疗银屑病和多发性银屑病硬化症。DMF 主要调节 Nrf2 和 NF-κB 细胞信号通路。在本研究中，我们采用氧糖剥夺/复氧（OGD/R）心肌模型用 H9c2 心肌细胞评价 DMF 的潜在保护作用。我们发现 DMF 能显著提高细胞活力，减少促炎细胞因子 IL-6、IL-8 的表达及趋化因子 MCP-1 的表达。我们进一步证明了 DMF 的抗氧化作用，其机制是通过抑制 NOX4 的表达减少 ROS 生成。组织因子 TF 和 ICAM-1 在左心室重塑中起主要作用，OGD/R 可诱导 TF 和 ICAM-1 的表达，DMF 通过抑制 Egr-1 信号通路抑制 OGD/R 诱导的 TF 和 ICAM-1 的表达，这些发现证明了 DMF 在心肌梗死治疗中的潜在作用。

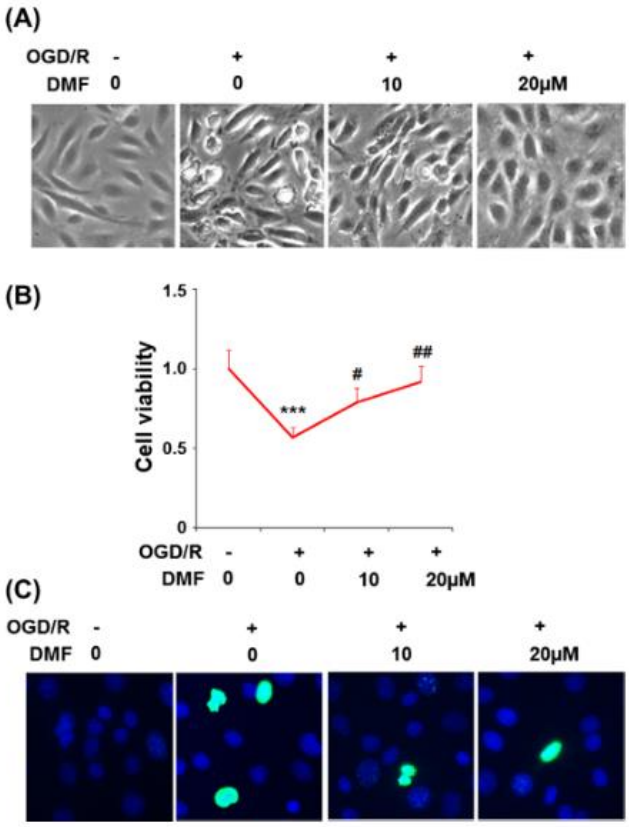


Fig2. Fig. 2. Dimethyl fumarate prevented oxygen-glucose deprivation/reperfusion (OGD/R)-induced reduction of cell viability in H9c2 cardiomyocytes. H9c2 cells were stimulated with dimethyl fumarate (10, 20 μM) for 6 h, followed by exposure to oxygen-glucose deprivation (6 h)/reperfusion (24 h) (OGD/R). (A).Morphology of H9c2 cells; (B). Cell viability of H9c2 cells was measured byMTT assay. (C). Apoptosis of H9c2 cells as measured by TUNEL assay. (***,P < 0.001 vs. vehicle group; #, ##, P < 0.05, 0.01 vs. OGD/R group).

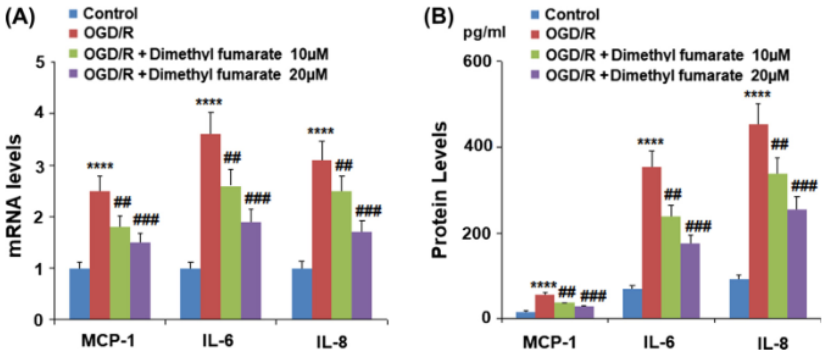


Fig. 3. Dimethyl fumarate reduced the expressi on and secretions of proinflammatory cytokine s and chemokines in H9c2 cardiomyocytes. H9 c2 cells were stimulated with dimethyl fumarat e (10, 20 μM) for 6 h, followed by exposure to oxygen-glucose deprivation (6h)/reperfusion (2 4 h) (OGD/R).(A). mRNA levels of MCP-1, IL -6, and IL-8 inH9c2 cells; (B). Secretions of M CP-1, IL-6,and IL-8 (****, P < 0.0001 vs. vehi clegroup; ##, ###, P < 0.01, 0.001 vs.OGD/R g roup).

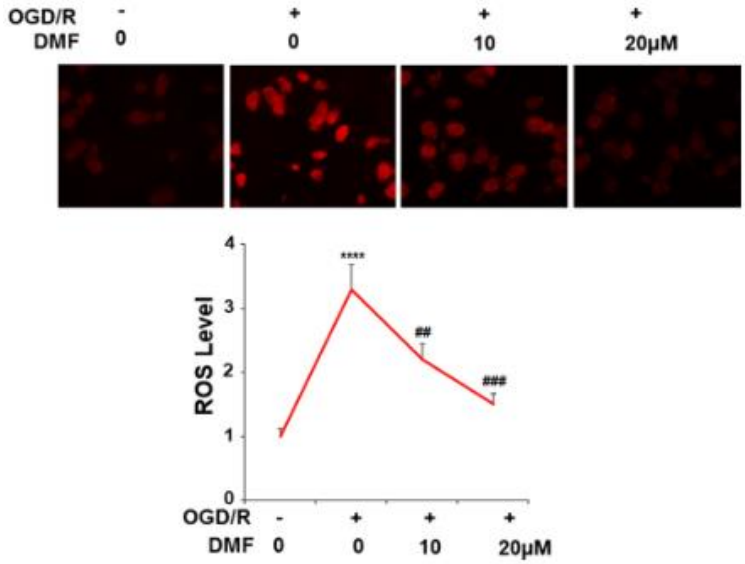
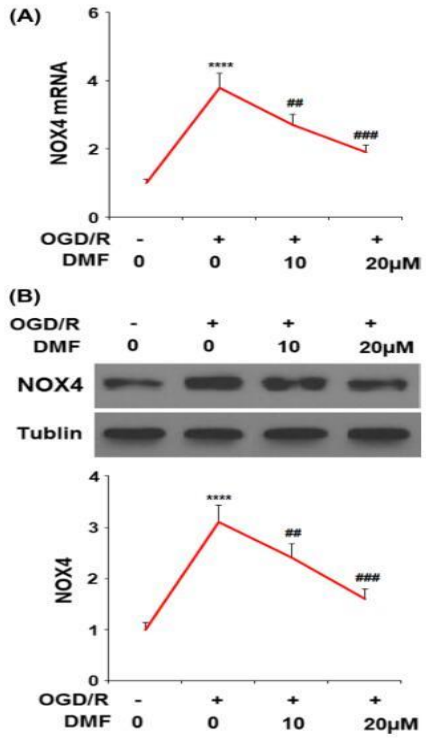


Fig. 4. Dimethyl fumarate prevented oxygen-glucose deprivation/reperfusion(OGD/R)-induced generatio n of reactive oxygen species (ROS) in H9c2 cardi-o myocytes. H9c2 cells were stimulated with dimethyl fumarate (10, 20 μM) for6 h, followed by exposure to OGD/R. Production of reactive oxygen species(R OS) was measured by dihydroethidium (DHE) staini ng (****, P < 0.0001 vs.vehicle group; ##, ###, P < 0.01, 0.001 vs. OGD/R group).



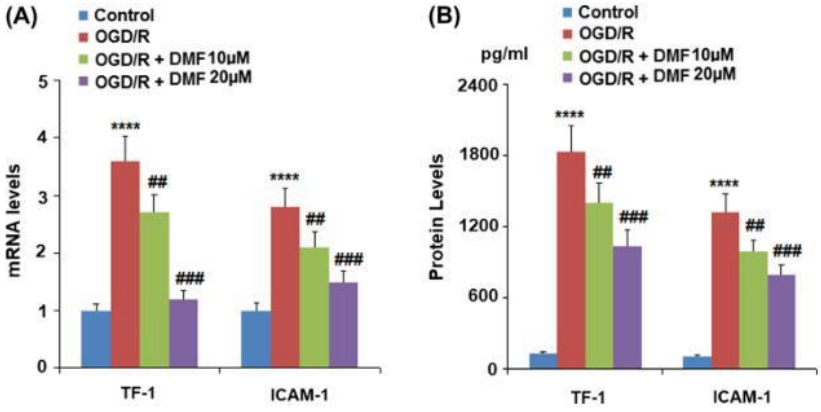


Fig. 6. Dimethyl fumarate reduced oxygen-glucose deprivation/reperfusion (OGD/R)-induced expression of ICAM-1 and tissue factor (TF) in H9c2 car-diomyocytes. H9c2 cells were stimulated with dimethyl fumarate (10, 20 μM) for 6 h, followed by exposure to OGD/R. (A). mRNA of tissue factor (TF) and ICAM-1; (B). Protein levels of tissue factor (TF) and ICAM-1 as measured by ELISA assay (****,P < 0.0001 vs. vehicle group; ##, ###,P < 0.01, 0.001 vs. OGD/R group).

Fig. 5. Dimethyl fumarate prevented oxygen-glucose deprivation/reperfusion (OGD/R)-induced oxidative stress in H9c2 cardiomyocytes. H9c2 cells werestimulated with dimethyl fumarate (10, 20 μM) for 6 h, followed by exposure to OGD/R. (A). mRNA of NOX4 as measured by real-time PCR; (B). Protein of NOX4 as measured by western blot analysis (****, P < 0.0001 vs. vehiclegroup; ##, ###, P < 0.01, 0.001 vs. OGD/R group).

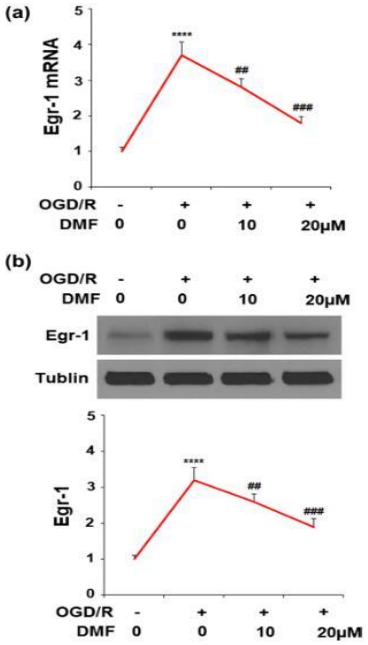


Fig. 7. Dimethyl fumarate reduced oxygen-glucose deprivation/reperfusion (OGD/R)-induced expression of Egr-1 in H9c2 cardiomyocytes. Cells were stimulated with dimethyl fumarate (10, 20 μM) for 6 h, followed by exposure to OGD/R. (A). mRNA of Egr-1; (B). Protein of Egr-1 (****, P < 0.0001 vs. vehicle group; ##, ###,P < 0.01, 0.001 vs. OGD/R group).

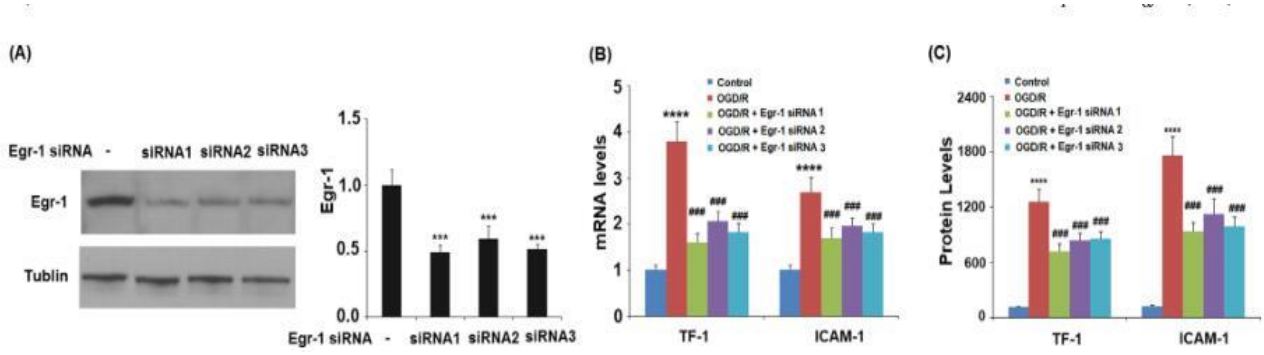


Fig. 8. Silence of Egr-1 prevented oxygen-glucose deprivation/reperfusion (OGD/R)-induced expression of ICAM-1 and tissue factor (TF) in H9c2 cardiomyocytes. H9c2 cardiomyocytes were transfected with Egr-1 siRNA or vehicle control siRNA, followed by exposure to OGD/R. (A). Western blot analysis revealed successfulknockdown of Egr-1; (B). mRNA of TF and ICAM-1; (C). Protein of TF and ICAM-1 as measured by ELISA (***, ****, P < 0.001, 0.0001 vs. vehicle group; ###,P < 0.001 vs. OGD/R group).

氧糖剥夺/复氧（OGD/R）可诱导 H9C2 大鼠心肌细胞发生凋亡，富马酸二甲酯（DMF）可抑制 OGD/R 诱导的细胞凋亡；DMF 可降低 OGD/R 诱导的 H9c2 心肌细胞促炎细胞因子和趋化因子的表达和分泌，并通过抑制 NOX4 的表达抑制 OGD/R 诱导的 H9c2 细胞 ROS 的生成；此外 DMF 降低 OGD/R 诱导的 ICAM-1 与 TF 的表达，抑制 OGD/R 诱导的 Egr-1 的表达，而沉默 Egr-1 同样抑制 OGD/R 诱导的 ICAM-1 与 TF 的表达。以上研究表明 DMF 通过抑制 NOX4 抑制 OGD/R 诱导的的氧化应激反应，并通过抑制 Egr-1 信号通路抑制 OGD/R 诱导的 TF 和 ICAM-1 的表达，进而改善 OGD/R 诱导的炎症反应和心肌损伤。