丙酮酸乙酯抑制脓毒症时 HMGB1 释放的分子机制

杨 超[1], 吴传新[1], 孙 航[2*], 龚建平[1], 刘 杞[2]
(重庆医科大学 1.附属第二医院肝胆外科,2.病毒性肝炎研究所, 重庆 400010)

摘 要:【目的】探讨丙酮酸乙酯(EP)抑制脓毒症巨噬细胞表达和释放 HMGB1 的相关分子机制。【方法】将小鼠腹腔巨噬细胞株 RAW264.7 分为 LPS 和 LPS+ EP 组,分别采用 100 ng/mL LPS 和 100 ng/mL LPS+ 5 mmol/L EP 刺激,于刺激后不同的时间点,Western blot 检测细胞总蛋白内 p-p38MAPK、CBP 的含量变化以及胞质和胞核内 NF-κB 的含量; 用免疫细胞化学、激光共聚焦显微镜观察培养细胞内 p-p38MAPK、NF-κB 和 CBP 的变化;Real-time PCR 检测培养细胞 HMGB1 的 mRNA 水平,ELISA 检测培养上清 HMGB1 的蛋白含量。【结果】LPS 和 LPS+ EP 刺激后 2~6 h,细胞内 p-p38MAPK 蛋白含量逐渐增加,但 LPS+ EP 组 p-p38MAPK 蛋白含量明显低于 LPS 组;NF-κB 在细胞质内的含量逐渐减少,而在细胞核内的含量逐渐增多,而 LPS+ EP 组 NF-κB 从胞质到胞核的移位明显弱于 LPS 组;细胞内 CBP 的蛋白含量逐渐增加,但 LPS+ EP 组明显低于 LPS 组。随着 p-p38MAPK、NF-κB 和 CBP 等蛋白的变化,LPS 和 LPS+ EP 刺激后 24、36 和 48 h,LPS+ EP 组细胞内 HMGB1 mRNA 表达比 LPS 组明显减少; 在 LPS 和 LPS+ EP 刺激后 18~48h,LPS+ EP 组培养上清中 HMGB1 蛋白含量明显低于 LPS 组。【结论】EP 通过抑制单核-巨噬细胞内的信号分子 p-p38MAPK、NF-κB、和 CBP 的表达,从而抑制 LPS 诱导单核-巨噬细胞表达和释放 HMGB1。

关键词:高迁移率族蛋白 B1;脓毒症;丙酮酸乙酯;分子机制
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Research Molecular Mechanisms of Inhibition Releasing of HMGB1 by Ethyl Pyruvate in Sepsis

YANG Chao[1], WU Chuan-xin[1], SUN Hang[2*], GONG Jian-pin[1], LIU Qi[2]
(1.Department of Hepatobiliary Surgery, The Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010, China; 2.Key Laboratory of Molecular Biology for Infectious Diseases, Ministry of Education, Liver Diseases Research and Treatment Center, Chongqing 400010, China)

Abstract:【Objective】To investigate the molecular mechanisms about ethyl pyruvate (EP) inhibit the expression and releasing of HMGB1 by macrophages in sepsis.【Methods】The murine macrophage-like cell line RAW264.7 cultured in vitro divided into LPS group and LPS +EP group. These groups were stimulated with 100 ng/mL LPS and 100 ng/mL LPS mixed with 5 mmol/L EP respectively. Western blot was used to detect the expression of protein in the cells about p-p38MAPK, CBP, NF-κB in nucleus and NF-κB in cytoplasm at different time-points. Immunocytochemistry and confocal laser scanning microscopy were used to confirm the change of p38-MAPK, NF-κB, and CBP in cultured cells. The expression of HMGB1 mRNA in cultured cells was determined by Real-time PCR. The content of HMGB1 protein in cultured cells supernatant were detected by ELISA.【Results】After stimulated by LPS and LPS+EP respectively from 2 to 6 hour, the protein level of p-p38MAPK in cells while this increase group of LPS+EP was obviously slower than the group of LPS. The expression of NF-κB protein in cytoplasm reduce while the same factor in nuclear increase gradually and this phenomenon was more weaker in LPS+EP group than that in LPS group. The protein of CBP in cells gradually increase while the protein of CBP in LPS+EP group was lower than that in LPS group. With variation of p38-MAPK, NF-κB, and CBP, the expression of HMGB1 mRNA in the cells of LPS group was decreased significantly than the ones in LPS group after each group stimulated with LPS and LPS+EP 24 h, 36 h, and 48 h, respectively. The content of HMGB1 protein in cultured cells supernatant of LPS+EP group was obviously lower than that of LPS group at the same time points after separately stimulated by LPS+

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作者简介:杨超,外科学硕士研究生,研究方向:炎症及肿瘤相关因子及信号通路,E-mail:cqydyc@gmail.com; * 通信作者:孙航,博士,副主任技师,E-mail:sunhang-wu@21cn.com