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葛根素预处理对LPS诱导RAW264.7细胞活化的影响

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中文摘要:目的:观察葛根素预处理对脂多糖(LPS)诱导小鼠巨噬细胞RAW264.7活化和分泌细胞因子的影响,探讨其抗炎机制。方法:取对数期生长良好的 RAW264.7细胞,随机分为空白对照组、LPS 组、葛根素预处理+LPS组。CCK-8法检测葛根素对RAW264.7的细胞毒性作用,Giemsa染色法观察细胞形态学变化,酶联免疫吸附试验(ELISA)检测细胞上清中肿瘤坏死因子-α(TNF-α)、巨噬细胞炎性蛋白-2(MIP-2)的变化,实时荧光定量PCR(qRT-PCR)动态测定NF-κB p65 mRNA的表达。 结果:当葛根素的浓度为100,200,400 μmol·L^{-1}时,与 1 mg·L^{-1} LPS共培养均未显示出细胞毒性作用(P<0.05);与空白对照组相比,LPS组可明显改变RAW264.7 细胞的形态(胞体增大,形态不规则,伪足大量伸出),葛根素100 μmol·L^{-1}组干预后可明显抑制LPS导致的细胞形态学变化,葛根素200,400 μmol·L^{-1}干预组的抑制效果更显著,但2组之间无明显差异;葛根素预处理可使细胞上清液中TNF-α,MIP-2以及细胞内NF-κB p65 mRNA的表达受到抑制(P<0.05),并随着葛根素浓度的增加,抑制效果逐渐增加(P<0.05),但未达到对照组水平。 结论:葛根素是一种安全有效的天然抗炎药物,可显著下调炎症细胞因子(如TNF-α,MIP-2)的表达,其作用机制可能与下调NF-κB p65 mRNA的表达有关。

中文关键词:葛根素 巨噬细胞 脂多糖 NF-κB

Effect of pretreatment with puerarin on activation of LPS-induced RAW264.7 cells

Abstract:Objective: To observe the effect of pretreatment with puerarin on activation of LPS -induced RAW264.7 cells and secretory cytokines, and discuss its anti-inflammatory mechanism. **Method:** Well-grown RAW264.7 cells in the exponential phase were collected and randomly divided them into the blank control group, the LPS group and the puerarin pretreatment+LPS group. The cellular toxic effect of puerarin on RAW264.7 cells was examined by CCK-8 assay, cell morphology was detected by Giemsa stain method, the changes in TNF-α and MIP-2 were tested by ELISA, and the expression of NF-κB p65 mRNA were determined by qRT-PCR. **Result:** When puerarin was cultured with 1 mg·L^{-1} LPS at a concentration of lower than 400 μmol·L^{-1}, it had not showed the cellular toxic effect (P<0.05). Compared with the control group, the LPS group could significantly change the morphology of RAW264.7 cells (increase in cell body, irregular shape, with a large number of pseudopodia extending). After intervention, the puerarin 100 μmol·L^{-1} group could significantly inhibit LPS-induced cell morphological changes, while the puerarin 200 μmol·L^{-1} and 400 μmol·L^{-1} puerarin groups showed more notable inhibitory effects. However, there was no obvious difference between the two groups. The pretreatment with puerarin could inhibit the expression of TNF-α and MIP-2 in cell supernatant and NF-κB p65 mRNA in cells (P<0.05). With increase in the puerarin concentration, its inhibitory effect gradually grew (P<0.05), but did not reach the level of the blank control group. **Conclusion:** As a safe and effective natural anti-inflammatory drug, puerarin can significantly reduce the expression of inflammatory cytokines (TNF-α, MIP-2). Its mechanism may be related to the reduction of NF-κB p65 mRNA expression.

keywords: puerarin RAW264.7 cell lipopolysacchride (LPS) NF-κB

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