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论著

LPS对金属蛋白酶的诱导表达及中药热毒清的保护机制

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摘要 背景与目的： 以肿瘤坏死因子(Tumour necrosis factor-α,TNF-α)前体向分泌型TNF-α转化过程为目标，探讨HL-60细胞中金属蛋白酶(Metalloproteinases, MPs)家族的基质金属蛋白酶(Matrix metalloproteinases, MMPs)和解整合素金属蛋白酶(A disintegrin and metalloproteases, ADAM)两亚类成分参与炎症因子分泌的机理以及中药热毒清(Reduqing, RDQ)抗炎保护功效的分子机制。 材料与 方法： 采用细胞毒性-MTT检测法、原位杂交、PAG-底物(明胶)电泳等方法分别检测HL-60、U937和小鼠腹腔巨噬细胞在大肠杆菌内毒素(LPS)及RDQ的作用下，MMPs、ADAM17、TNF-α基因转录水平和酶活性的变化。 结果： ①LPS刺激后，不同类型细胞的MMPs的表达量及电泳酶谱随刺激时间的延长变弱，而HL-60细胞培养上清的酶谱变化正好相反；②在HL-60细胞中与MMPs相比，ADAM17在前体TNF-α (pro-TNF-α)向分泌型TNF-α (s-TNF-α)转换中起着重要作用；③RDQ在细胞杀伤效应上、在ADAM17 mRNA表达水平上均能明显抑制LPS所诱导的s-TNF-α表达和分泌的增高作用(P<0.01)。 结论： ①在LPS刺激的TNF-α分泌中，MPs家族的ADAM17起主要作用，而针对解整合素结构域(Disintegrin)的探针对排除MMPs的干扰，真实反映ADAM17 mRNA表达水平更具特异性；②纯中药注射液RDQ的抗炎、解毒作用的靶标之一是拮抗LPS诱导的TACE mRNA活性增加。ADAM17成为炎症机制和治疗研究的新靶标。

关键词 基质金属蛋白酶； 肿瘤坏死因子转换酶； 肿瘤坏死因子； 大肠杆菌内毒素； 热毒清

The Induced Expression of Metalloproteinases by LPS and the Protection Mechanism of Chinese Herb "Reduqing"

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Abstract BACKGROUND & AIM: To study the function and role of two subclasses in MPs family, MMPs and ADAMs, for processing of precursor TNF-α, and explore the anti-inflammatory mechanisms of Chinese herbal preparation Reduqing (RDQ). MATERIAL AND METHODS: The in vitro study was carried out on HL-60、U937 cells and macrophages of murine abdominal cavity treated by LPS and RDQ. Using MTT colorimetry,in situ hybridization and Gelatin-PAGE to detect transcriptional levels of MMPs,ADAM17, TNF-α mRNA and the changes of enzymatic activity. RESULTS: ①After stimulation by LPS, the expression level of MMPs and electrophoresis zymogram on gelatin-PAGE decreased with increased stimulation time; ②ADAM17 (TACE) played a more important role in precursor processing of proTNF-α of HL-60 cells compared with MMPs; ③RDQ had obvious inhibitory effects on enhancing secretion of TNF-α induced by LPS stimulation (P<0.01) at the transcriptional level of ADAM17 mRNA in HL-60 cells. CONCLUSION: ①ADAM17 of MPs family had a main role in TNF-α secretion induced by LPS. It is a key step to choose and design the correct probe or primers aiming specifically at the disintegrin domain for ADAM17, in order to eliminate the interference of MMPs and reflect the true expression level of ADAM17 gene; ②The mechanism of RDQ against inflammation may be its inhibitory effect on ADAM17 mRNA expression activated by LPS. ADAM17 is able to represent the novel target for studing the mechanism and the

therapeutic pathway for inflammation.

Keywords matrix metalloproteinases TNF-α Lipopolysacharides Reduging

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