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论文

ISO-1对人脐静脉内皮细胞Toll样受体4活性的调节及意义

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摘要：

目的 探讨巨噬细胞移动抑制因子（MIF）特异抑制剂ISO-1［(S,R)-3-(4-羟苯基）-4,5-二氢-5-异噁唑乙酸，甲酯 ］是否通过Toll样受体4（TLR4)途径影响人脐静脉内皮细胞（HUVEC）活性，从而为ISO-1用于TLR4相关疾病的靶向治疗奠定基础。方法 将传代培养的HUVEC分为脂多糖（LPS）组、ISO-1预处理组、LPS+ISO-1组和培养液对照组，通过RT-PCR、免疫荧光染色、放射免疫分析和化学反应法，检测ISO-1对HUVEC TLR4表达和肿瘤坏死因子α（TNFα）、一氧化氮合酶（NOS）产生的影响。结果 高浓度ISO-1预处理HUVEC,可明显抑制TLR4mRNA 表达，其中以25和50μ mol/L浓度组最为明显(P＜0.05)；用50μmol/L ISO-1分别预处理HUVEC 0.5、1、2、3h，均能明显降低TLR4mRNA表达，其中1h组的作用最为明显(P＜0.05)。免疫荧光染色结果显示，HUVEC可表达低水平TLR4，LPS(10ng/mL)可促进TLR4表达，而50μmol/L的ISO-1预处理1h，可明显降低TLR4表达和LPS诱导的HUVEC TNFα分泌和NOS的产生（P＜0.05）。ISO-1预处理对HUVEC产生TNFα和NOS的抑制呈现浓度依赖性。ISO-1作用时间短暂，预处理1h组的作用效果最好（P＜0.05），此后逐渐减弱。结论 ISO-1可通过下调TLR4影响HUVEC功能，该结果可为ISO-1用于动脉粥样硬化和炎症等TLR4相关疾病的治疗提供实验依据。

关键词： 脂多糖；Toll样受体4；巨噬细胞移动抑制因子；脐静脉内皮细胞

Regulation of Toll-like receptor 4 by ISO-1 in HUVECs

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Abstract:

Objective To study the effect of ISO-1 ［(S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester］, a specific antagonist of MIF (Macrophage migration inhibition factor), on TLR4 (Toll-like receptor) activities in HUVEC (Human umbilical vein endothelial cells), and to provide an experimental evidence for the treatment of TLR4-related diseases in which ISO-1 is targeted at. Methods HUVECs were cultured and divided into 4 groups: LPS (lipid polysaccharide) group, ISO-1 pretreatment group, LPS+ISO-1 group and Medium control group. Effects of ISO-1 on TLR4 expression, TNFα (tumor necrosis factor) and NOS (nitric oxide synthase) secretion were investigated with RT-PCR, immunofluorescense staining, radioimmunoassay and enzyme-chemical reactions. Results The expression of TLR4 in HUVEC was obviously inhibited by pretreatment with high concentration of ISO-1, especially in 25 and 50μmol/L groups (P＜0.05). Low expression of TLR4 was also detected at 0.5, 1, 2 and 3h after the pretreatment with 50μmol/L ISO-1, in which the lowest appeared at 1h (P＜0.05) . Immunofluorescense showed low level TLR4 in normal HUVEC, however, the expression could be enhanced by LPS (10ng/mL). TLR4 expression, TLR4-induced TNFα and NOS secretion were markedly suppressed 1h later after the pretreatment with 50μmol/L ISO-1, and such inhibition was does-dependent (P＜0.05). The effective time of ISO-1 was short and the maximal was at 1h. Conclusion ISO-1 can exert the inhibitory effects through down-regulating the TLR4 activities of HUVEC, which suggests that ISO-1 may be a potential target-agent in the treatment of TLR4-related diseases, such as atherosclerosis and inflammation.

Keywords: Lipid polysaccharide; Macrophage migration inhibition factor; Toll-like receptor; Human umbilical vein endothelial cells

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