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World Chin J Digestol 2003 September;11(9):1396-1398
世界华人消化杂志 ISSN 1009-3079 CN 14-1260/R

胰腺移植物ICAM-1的表达及信号转导的因素

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辽宁省科委攻关课题资助项目, No. 00225001
辽宁省教育厅高校科研项目, No. 202012014.
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收稿日期: 2003-03-08 接受日期: 2003-03-25

Intercellular adhesion molecule-1 expression in pancreas graft and its signal transducer

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Supported by the Society Development Foundation of Liaoning Province, No. 99225003 and the Key Programs of Science and Technology Commission of Liaoning Province, No.00225001 and Fund from the Higher Education Department of Liaoning Province, No.202012014
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Received: 2003-03-08 Accepted: 2003-03-25

Abstract

AIM: To investigate the effect of neutrophil elastase inhibitor (ONO-5046) on expression of intercellular adhesion molecule-1 and transduction signal after pancreasduodenal transplantation in rats.

METHODS: ONO-5 046 was injected intravenously into experimental animal models. ICAM-1 mRNA transduction signals were detected in rat endothelial cells with regard to the effect of many reagents on expression of ICAM.

RESULTS: ICAM-1 mRNA level decreased in pancreatic grafts of experimental animals. ICAM-1 mRNA expression was increased in rat endothelial cells *in vitro* stimulated by NE, while that it could be inhibited by ONO-5046. Calcium ionophore enhenced ICAM-1 mRNA expression. In contrast, a phospholipase C inhibitor, calcium chelator and nuclear factor-kappa B inhibitor regulated down NE induction of ICAM-1 mRNA.

CONCLUSION: ICAM-1 expression stimulated by NE in pancreatic grafts may be associated with intracellular Ca^{2+} influx and a phospholipase C signal transduction.

Liang J, Wang FS, Liu YF, Liu LM, Liu SR, Cui H, Tai CQ, He SG. Intercellular adhesion molecule-1 expression in pancreas graft and its signal transducer. Shijie Huaren Xiaohua Zazhi 2003;11(9):1396-1398

摘要

目的: 探讨中性粒细胞弹性蛋白酶(NE)抑制剂对细胞间黏附因子-1 (ICAM-1)在大鼠胰腺移植物的表达及转导信号的影响.

方法: 采用大鼠胰十二指肠移植模型, 实验组给予NE抑制剂(ONO-5046, 10 mg/kg). 体外实验检测NE及多种相关试剂对大鼠内皮细胞ICAM-1mRNA表达的影响及基因转导信号的调控作用.

结果: 对照组胰腺移植物中ICAM-1mRNA呈高水平表达, 而实验组经ONO-5046处理后明显下调其表达, 有显著性差异. NE刺激大鼠内皮细胞上调ICAM-1mRNA表达水平, 而ONO-5046则明显抑制其表达; 特异性钙离子载体增强该细胞的ICAM-1mRNA表达, 相反, 磷脂酶C抑制剂、钙离子螯合剂及核因子κB抑制因子则下调NE诱导的ICAM-1mRNA表达水平.

结论: NE增强ICAM-1在胰腺移植物的表达与细胞钙离子内流及磷脂酶C的信号转导有关.

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http://www.wjgnet.com/1009-3079/11/1396.asp

0 引言

胰腺移植物的再灌注损伤性胰腺炎和移植物血栓形成是胰腺移植早期移植物失功的主要并发症. 中性粒细胞在缺血/再灌注损伤过程中起重要作用, 中性粒细胞弹性蛋白酶(NE)是炎症组织损伤的一个重要递质, 而巨噬细胞分化抗原-1 (Mac-1)和细胞间黏附因子-1 (ICAM-1)等炎症因子也起着重要作用[1-9]. 我们曾报道NE抑制剂降低大鼠胰十二指肠移植再灌注后中性粒细胞趋化因子的表达[5]. 本研究探讨大鼠胰十二指肠移植再灌注损伤过程中ICAM-1在胰腺移植物的表达以及ICAM-1mRNA基因信号转导的调控因素.

1 材料和方法

1.1 材料 ♂ Wistar大鼠, 体质量250-300 g, 采用Lee

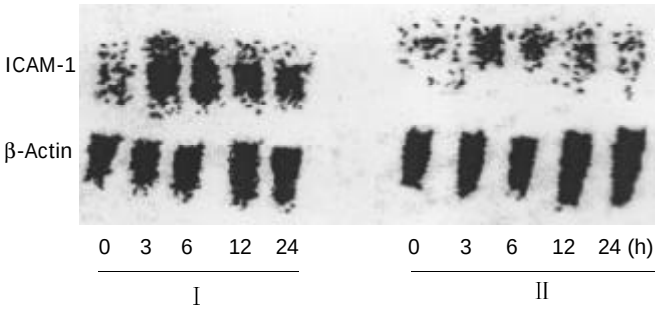
法大鼠异位胰十二指肠移植术. 实验组移植物血管开放前静脉给予弹性蛋白酶抑制剂(ONO-5046)10 mg/kg，对照组静脉给予等量生理盐水. 胰腺移植后3，6，12，24 h各组分别处死4只动物，取血及胰腺移植物标本置低温冰箱保存待测. 特异性钙离子载体(A_{23187}) 2 μmol/L (Sigma); 磷脂酶C抑制剂(U_{73122}) 5 μmol/L (Biomol); 内质网钙释放阻滞剂(TMB-8) 50 μmol/L (Biomol); 核转录因子-κB抑制剂(PDTC) 10 μmol/L (Sigma); NE抑制剂(ONO-5046) 10 mmol/L (日本熊本大学山口康雄副教授惠赠).

1.2 方法 免疫组化染色采用碱性磷酸酶法对胰腺移植物行ICAM-1免疫组化染色，应用图像分析仪(METAMORPH，USA)进行半定量分析(标准化单位). 通过预实验发现，不同浓度的NE刺激WK-5细胞后ICAM-1mRNA的表达水平呈剂量依赖性增加. 然而，高浓度NE (10 mg/L)则导致WK-5细胞从细胞培养平板上剥离. 故采用NE 5 mg/L刺激WK-5细胞进行体外实验. 在RPMI 1640加100 ml/L FCS液中调整WK-5细胞浓度为10^6/ml，於24孔培养板中，在50 ml/L CO_2，37 ℃条件下培养24 h. 对胰腺移植物及WK-5细胞行RNA分离提取和Northern blot分析，检测ICAM-1mRNA的表达.

统计学处理 实验数据以$\bar{x} \pm s$表示，采用统计学软件包对结果进行方差分析和t检验，判断各组间差异显著性.

2 结果

移植后24 h胰腺移植物的免疫组化染色显示，对照组的ICAM-1表达明显高于ONO-5046预处理的实验组，彩色图像分析半定量结果，ICAM-1在对照组胰腺移植物中的表达值为82 ± 11，明显高于实验组的38 ± 9，两组间有显著性差异(P<0.01). Northern blot分析，对照组胰腺移植物ICAM-1mRNA表达水平明显增高，移植后3 h达高峰，然后逐渐降低. 而实验组胰腺移植物的ICMA-1mRNA表达水平明显降低(P<0.01，图1). NE



Ⅰ 对照组; Ⅱ 实验组.
图1 胰腺移植物中ICAM-1mRNA的表达.

加入WK-5细胞培养液后明显上调ICAM-1mRNA表达水平，而ONO-5046则明显抑制其表达，两组间有显著性差异(P<0.01，图2). NE加入WK-5细胞培养液后ICAM-1mRNA表达水平(相对密度2.24 ± 0.21)，特异性钙离子载体(A_{23187})增强NE刺激WK-5细胞的ICAM-1mRNA表达水平(相对密度2.82 ± 0.17)，相反，磷脂酶C抑制剂(U_{73122})则下调其表达(相对密度0.91 ± 0.24)，而内质网钙释放阻制剂(TMB-$_8$)和核转录因子-κB抑制剂(PDTC)明显抑制NE刺激WK-5细胞的ICAM-1mRNA表达(相对密度分别为0.20 ± 0.03、0.17 ± 0.02)，ONO-5046同样抑制NE刺激WK-5细胞的ICAM-1mRNA表达(相对密度0.84 ± 0.16，图3).

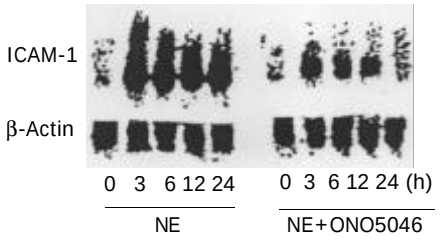
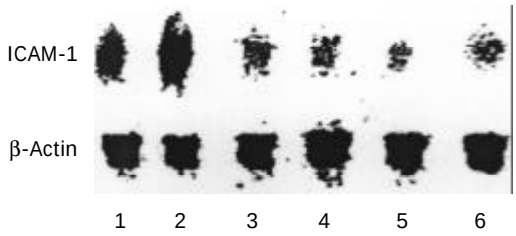


图2 WK-5细胞的ICAM-1mRNA表达.



1 NE; 2 A_{23187} +NE; 3 U_{73122} +NE; 4 TMB-8+NE; 5 PDTC+NE; 6 ONO5046+NE.
图3 不同试剂对WK-5细胞ICAM-1mRNA表达的影响.

3 讨论

本实验证明NE抑制剂ONO-5046明显抑制大鼠胰十二指肠移植再灌注后胰腺移植物中ICAM-1蛋白和基因的表达. 有报道，ONO-5046对犬心脏移植的缺血再灌注损伤亦有保护作用[10]. 人们从不同角度进行研究取得了满意的成果[11-14]. 本研究体外实验提示，NE上调内皮细胞ICAM-1mRNA的表达水平. 受体-G蛋白-磷脂酶C(PLC)复合物被膜磷脂酰肌醇(PI)酶切形成第2信使二酯甘油(DG)和三磷酸肌醇(IP_3). 由PLC作用的IP_3水解可通过刺激受体或通过Ca^{2+}通道的开放来调节[15, 16]. 细胞间IP_3和Ca^{2+}浓度的增加可被PLC抑制剂U_{73122}剂量依赖性抑制. 弹性蛋白酶增强Ca^{2+}内流，从而导致PLC的激活. 这些结果提示，在弹性蛋白酶的作用下，ICAM-1的表达可能与细胞Ca^{2+}内流和磷脂酶C的激活有关. 本实验通过弹性蛋白酶刺激大鼠内皮细胞，探讨ICAM-1基因表达的信号转导机制.

实验结果提示: NE明显增加内皮细胞的ICAM-1mRNA表达，而NE抑制剂ONO-5046则抑制其表达; Ca^{2+}特异性载体A_{23187}上调内皮细胞ICAM-1mRNA的表达，而Ca^{2+}螯合剂TMB-8则明显下调其表达; PLC的活性是Ca^{2+}依赖性的，PLC抑制剂U_{73122}可以抑制由NE刺激内皮细胞的ICAM-1mRNA水平的增高，证明PLC参与调节ICAM-1基因启动子在大鼠内皮细胞表

达的信号转导且与Ca^{2+}内流密切相关. 当白细胞、单核细胞表面的黏附因子(Mac-1)与细胞外基质结合后，可激活细胞内的信号转导途径，细胞内Ca^{2+}浓度增高是白细胞迁移所必需的，而内皮细胞表面的ICAM-1介导了白细胞穿越毛细血管壁达到炎症损伤部位的黏附和浸润过程[17]. 抑制蛋白酶活性对防止中性粒细胞迁移以及局部或远隔器官损伤是有效的[18]. 缺血、细胞内钙超载是移植物失功的重要因素[19]. 实验研究发现，缺氧使移植物细胞内ATP耗竭，导致细胞内钠、钾、钙离子紊乱[20].

胰腺缺血再灌注损伤可导致微循环障碍[21]. 乏氧、缺血再灌注损伤可通过酪氨酸磷酸化依赖性通道活化NF-κB[22]，后者可与多种炎症因子基因启动子的κB序列结合，参与炎症递质、黏附因子基因的转录. 这些炎症递质可以活化内皮细胞、单核巨噬细胞、白细胞，释放氧自由基、NE等物质进一步加重组织损伤. NF-κB介导多种免疫调节因子的表达，IκBα是NF-κB的主要抑制因子. 增加细胞间钙离子信号传导通路能激活Ca^{2+}依赖性蛋白酶，他使IκBα蛋白溶解[23-29]. ICAM-1基因5'调节区域有NF-κB结合位点，信号转导因子可与之结合而影响ICAM-1基因的转录. 本研究证明NF-κB抑制剂PDTC明显抑制NE诱导的ICAM-1mRNA表达水平. NE的蛋白分解增加了内皮细胞的通透性，并且是内皮细胞变形和分离的最有效的蛋白酶之一. 在大鼠胰腺移植缺血再灌注后早期NE明显增强IL-8/CINC mRNA表达，并上调ICAM-1在内皮细胞的表达.

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