

论著

去甲斑蝥素对高糖刺激的HK-2 细胞FN,Col IV 和TGF- β 1 mRNA 及蛋白表达的影响

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摘要:

目的: 观察去甲斑蝥素(NCTD) 对高糖刺激的HK-2 细胞外基质和TGF- β 1 表达的影响。方法: 常规培养HK-2 细胞, 无血清DMEM 培养基同步培养24 h, 细胞分为正常葡萄糖组(C, *D*-glucose 5.5 mmol/L)、甘露醇对照组(M, 5.5 mmol/L *D*-glucose+24.5 mmol/L mannitol)、高糖组(HG, 30 mmol/L *D*-glucose)、高糖+NCTD 干预组(30 mmol/L *D*-glucose+0.5~40 mg/L NCTD)。台盼蓝排斥实验检测NCTD 对高糖刺激的细胞毒性。MTT 法检测NCTD 对高糖刺激的细胞增殖的影响。收集培养6, 24, 48 h 后细胞总RNA 及蛋白, 用RT-PCR 检测细胞FN, Col IV 和TGF- β 1 mRNA 的表达, 采用Western 印迹检测FN, Col IV 和TGF- β 1 蛋白的表达。结果: 不同浓度NCTD 作用72 h 后, 浓度超过5 mg/L 的NCTD 对高糖环境下的HK-2 细胞有明显的毒性。RT-PCR 和Western 印迹结果显示: 30 mmol/L *D*-葡萄糖可引起HK-2 细胞FN, Col IV 和TGF- β 1 mRNA 及蛋白水平的升高($P < 0.05$), 而5 mg/L NCTD 可抑制高糖刺激的FN, Col IV 和TGF- β 1 的表达($P < 0.05$)。相同渗透浓度的*D*-甘露醇组对上述指标均无影响($P > 0.05$)。结论: NCTD 能下调HK-2 细胞FN, Col IV 及TGF- β 1 的表达。

关键词: 去甲斑蝥素 纤粘连蛋白 胶原IV 转化生长因子- β 1

Effect of norcantharidin on the expression of FN, Col IV and TGF- β 1 mRNA and protein in HK-2 cells induced by high glucose

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Abstract:

Objective: To observe the effect of norcantharidin (NCTD) on the expression of mRNA and protein of fibronectin (FN), collagen IV(Col IV) and transforming growth factor- β 1(TGF- β 1) in human kidney proximal tubular epithelial (HK)-2 cells induced by high glucose.

Methods: HK-2 cells were incubated with serum-free DMEM for 24 h to synchronize cell growth, and then the cells were divided into 4 groups: Group C (5.5 mmol/L *D*-glucose), Group M (5.5 mmol/L *D*-glucose + 24.5 mmol/L-mannitol), Group HG (30 mmol/L *D*-glucose), and Group HG + NCTD (30 mmol/L *D*-glucose + 0.5-40 mg/L NCTD). Cytotoxicity of HK-2 cells induced by high glucose of NCTD was detected by Trypan blue dye exclusive assay. The effect of NCTD on the proliferation of HK-2 cells in high glucose was determined by MTT. The cells were collected to extract total RNA and protein at 6, 24 and 48 h after the incubation. The expression of FN, Col IV and TGF- β 1 mRNA was examined by RT-PCR, and FN, Col IV and TGF- β 1 protein was analyzed by Western blot.

Results: Trypan blue dye exclusive assay showed NCTD concentrations over 5 mg/L were rather toxic in HK-2 cells. The proliferation of HK-2 cells in high glucose was interrupted by interfered with 5 mg/L NCTD as measured by MTT ($P < 0.05$). NCTD at 5 mg/L had a stronger inhibitory effect than NCTD at 2.5 mg/L. Real-time PCR and Western blot showed that the mRNA and protein expression of FN, collagen IV and TGF- β 1 increased in HK-2 cells treated with high glucose ($P < 0.05$), while that in cells treated by NCTD was dramatically inhibited ($P < 0.05$). No change in these parameters was detected in the 30 mmol/L *D*-mannitol control group ($P > 0.05$).

Conclusion: NCTD can downregulate FN, collagen IV and TGF- β 1 mRNA and protein expression in HK-2 cells stimulated by 30 mmol/L *D*-glucose.

Keywords: norcantharidin fibronectin collagen IV TGF- β 1

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