

论著

法舒地尔对高糖诱导人肾小管上皮细胞转分化的影响

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收稿日期 2013-3-4 修回日期 2013-8-2 网络版发布日期 2013-10-18 接受日期

摘要 目的 探讨法舒地尔对高糖诱导的人肾小管上皮细胞(HK-2)转分化的影响及可能的作用机制。方法 HK-2细胞分别加入葡萄糖5.5 mmol·L⁻¹、葡萄糖5.5 mmol·L⁻¹+甘露醇54.5 mmol·L⁻¹、葡萄糖60 mmol·L⁻¹(高糖)以及葡萄糖60 mmol·L⁻¹+法舒地尔5, 10和20 μmol·L⁻¹。免疫共沉淀法检测葡萄糖60 mmol·L⁻¹作用0~24 h后磷酸化肌球蛋白磷酸酶目标亚单位1-苏氨酸696(p-MYPT1-Thr 696)和p-MYPT1-Thr 853的表达,以评估Rho相关的卷曲螺旋形成的蛋白激酶(ROCK)的活性;免疫细胞化学法检测α-平滑肌肌动蛋白(α-SMA)表达;Western蛋白质印迹法检测E-钙黏素、波形蛋白和结缔组织生长因子(CTGF)蛋白表达。结果 与未加高糖刺激前比较,高糖培养3 h后,细胞p-MYPT1-Thr696表达明显增加,积分吸光度(IA)值由1.08±0.09增加到2.4±0.09(P<0.01);与未加高糖刺激前比较,高糖培养7 h后,细胞p-MYPT1-Thr853表达明显增加, IA值由0.57±0.01增加到1.45±0.14(P<0.01),表明高糖能导致HK-2细胞ROCK分子活化。与正常对照组相比,葡萄糖60 mmol·L⁻¹组HK-2细胞培养72 h后E-钙黏素表达减少(P<0.01),α-SMA、波形蛋白和CTGF表达增多(P<0.01);葡萄糖5.5 mmol·L⁻¹+甘露醇54.5 mmol·L⁻¹组与正常对照组比较无明显变化。与葡萄糖60 mmol·L⁻¹组相比,葡萄糖60 mmol·L⁻¹+法舒地尔5, 10和20 μmol·L⁻¹组E-钙黏素表达增多(P<0.01),α-SMA、波形蛋白和CTGF表达减少(P<0.01),且法舒地尔20 μmol·L⁻¹组改变更为明显,法舒地尔3个浓度组间比较差异有显著性(P<0.05)。结论 法舒地尔能抑制高糖诱导的肾小管上皮细胞转分化,可能部分通过减少CTGF的表达而产生作用。

关键词 法舒地尔 肾小管 上皮细胞 转分化 高糖

分类号 R966 R972.4

Effect of fasudil on epithelial-myofibroblast transdifferentiation of human renal tubular epithelial cells induced by high glucose

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Abstract

OBJECTIVE To investigate the effect of fasudil on the epithelial-myofibroblast transdifferentiation of human renal tubular epithelial (HK-2) cells induced by high glucose and to explore the mechanism. **METHODS** HK-2 cells were cultivated in glucose 5.5 mmol·L⁻¹, glucose 5.5 mmol·L⁻¹+mannitol 54.5 mmol·L⁻¹, high glucose (60 mmol·L⁻¹) and high glucose+fasudil 5, 10 and 20 μmol·L⁻¹, respectively, for 72 h. Changes in the p-MYPT1-Thr696 and p-MYPT1-Thr853 were detected with co-immunoprecipitation assay. α-Smooth muscle actin (α-SMA), which reflected the phenotypic characteristics of myofibroblast cells, was detected by immunocytochemistry. Western blotting was used to detect the protein expression of E-cadherin, vimentin and connective tissue growth factor (CTGF). **RESULTS** Compared with HK-2 cells without glucose 6.0 mmol·L⁻¹, the expression of p-MYPT1-Thr696 was enhanced after 3 h exposure to high glucose (integrated absorbance (IA) from 1.08±0.09 to 2.4±0.09, P<0.01), and that of p-MYPT1-Thr853 was enhanced after 7 h (IA from 0.57±0.01 to 1.45±0.14, P<0.01), suggesting that the activity of Rho kinase could be activated by high glucose. Compared with glucose 5.5 mmol·L⁻¹ group, HK-2 cells cultured with glucose 60 mmol·L⁻¹ showed a decreased expression of E-cadherin (P<0.01), increased expression of α-SMA, vimentin and CTGF (P<0.01). Compared with high glucose group, the high glucose+fasudil 5, 10 and 20 μmol·L⁻¹ groups showed an increased expression of E-cadherin (P<0.01), but decreased expression of α-SMA, vimentin and CTGF (P<0.01). The changes of fasudil 20 μmol·L⁻¹ group were the most obvious. **CONCLUSION** Fasudil can inhibit high glucose-induced epithelial-myofibroblast transdifferentiation of renal tubular epithelial cells, possibly by reducing the expression of CTGF.

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DOI: 10.3867/j.issn.1000-3002.2013.05.007

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