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论著

哇巴因对血管内皮细胞ECV304细胞凋亡及胞内游离钙离子和活性氧浓度的影响

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摘要 目的 研究哇巴因(毒毛花苷G)对人脐静脉血管内皮细胞ECV304凋亡的诱导作用，并探讨其可能的作用机制。方法 哇巴因0.01，0.05，0.1，0.5，1和10 μmol·L^{-1}与ECV304细胞作用24，48和72 h，MTT法检测细胞存活率，Hoechst33342/碘化丙锭双荧光染色法和流式细胞仪检测细胞凋亡百分率，激光共聚焦显微镜观察细胞内游离Ca^{2+}浓度([Ca^{2+}]$_i$)和活性氧(ROS)浓度，逆转录PCR和Western印迹法检测胱天蛋白酶3 mRNA和蛋白表达。结果 哇巴因在0.01~10 μmol·L^{-1}浓度范围内与ECV304细胞分别作用24，48和72 h，对细胞存活的抑制率明显增加，且呈浓度和时间依赖性，24，48和72 h浓度-效应相关系数分别为0.984，0.994和0.997(P<0.05)；哇巴因作用24，48和72 h的IC_{50}值分别为0.624，0.184和0.041 μmol·L^{-1}，时间-效应相关系数为0.974(P<0.05)。哇巴因0.1 μmol·L^{-1}与ECV304细胞作用24 h，细胞凋亡百分率由正常对照组的（4.2±0.5）% 升高到（26.0±3.2）%，作用48 h，细胞凋亡率由(4.7±0.5)%升高到(36.5±5.3)%，差异有统计学意义(n=3，P<0.01)；同时细胞出现染色质凝集。哇巴因0.01，0.1和0.5 μmol·L^{-1}分别与ECV304细胞作用12，24和36 h，[Ca^{2+}]$_i$和ROS浓度呈浓度和时间依赖性增加，在哇巴因0.5 μmol·L^{-1}时[Ca^{2+}]$_i$和ROS浓度的时间-效应相关系数分别为0.912和0.924，作用36 h时[Ca^{2+}]$_i$和ROS浓度的浓度-效应相关系数分别为0.889和0.907(P<0.05)。逆转录PCR和Western印迹法分析显示，哇巴因0.1和0.5 μmol·L^{-1}作用ECV304细胞24 h后，胱天蛋白酶3 mRNA表达增加，差异有统计学意义(P<0.05)；哇巴因0.01，0.1和0.5 μmol·L^{-1}作用ECV304细胞24 h，胱天蛋白酶3蛋白表达明显增加，差异有统计学意义(P<0.05)。结论 哇巴因可诱导人脐静脉血管内皮细胞ECV304凋亡，其机制可能与增加[Ca^{2+}]$_i$和ROS浓度及胱天蛋白酶3表达有关。

关键词 哇巴因 内皮细胞 细胞凋亡 钙 活性氧 胱天蛋白酶3

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Effect of ouabain on human vascular endothelial ECV304 cell apoptosis and on concentrations of intracellular free calcium ion and reactive oxygen species

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Abstract

OBJECTIVE To study the effect of ouabain (strophanthin G) on human vascular endothelial ECV304 apoptosis and the possible mechanism. **METHODS** ECV304 cells were treated with ouabain 0.01, 0.05, 0.1, 0.5, 1 and 10 μmol·L^{-1} for 24，48 and 72 h. The survival rates of cells were detected by MTT assay; cells morphological changes were studied by Hoechst 33342/propidium iodide staining; cell cycle distribution was detected by flow cytometry; and the concentrations of the intracellular free calcium ion ([Ca^{2+}]$_i$) and reactive oxygen species (ROS) in ECV304 cells were measured by the laser confocal microscope. The caspase 3 mRNA and protein expression level on ECV304 cells were detected by RT-PCR and Western blotting, respectively. **RESULTS** Ouabain 0.01-10 μmol·L^{-1} was incubated with ECV304 cells for 24，48 and 72 h. The inhibition rate of cell survival was increased in a concentration- and time-dependent manner. The coefficient of concentration-correlation was 0.984, 0.994 and 0.997(P<0.05), respectively. IC50 of ouabain at 24，48 and 72 h was 0.624, 0.184 and 0.041 μmol·L^{-1} and the coefficient of time-correlation was 0.974(P<0.05). Compared with normal control group, the apoptosis rate of ECV304 cells treated with ouabain 0.1 μmol·L^{-1}, from increased from （4.2±0.5）% to

（26.0±3.2）% at 24 h, and from (4.7±0.5)% to (36.5±5.3)% at 48 h, being statistically significant (n=3, P<0.01). The cells showed obviously defluxion and nuclear chromatin condensation when treated with ouabain 0.1 μmol·L^{-1} for 24 and 48 h. The ［Ca^{2+}］$_i$ and ROS concentration in ECV304 cells significantly increased in a concentration- and time-dependant manner after ECV304 cells were incubated with ouabain 0.01, 0.1 and 0.5 μmol·L^{-1} for 12, 24 and 36 h, respectively. When ouabain was 0.5 μmol·L^{-1}, the coefficient of time correlation of ［Ca^{2+}］$_i$ and ROS concentration were 0.912 and 0.924, respectively. When the ECV304 cells was incubated with ouabain for 36 h, the coefficient of concentration-correlation of ［Ca^{2+}］$_i$ and ROS concentration were 0.889 and 0.907 (P<0.05), respectively. The RT-PCR and Western blotting results showed that caspase 3 mRNA expression was up-regulated after ECV304 cells were treated with ouabain 0.1 and 0.5 μmol·L^{-1} for 24 h, being of statistical significance (P<0.05); caspase 3 protein expression was up-regulated after ECV304 cells were treated with ouabain 0.01, 0.1 and 0.5 μmol·L^{-1} for 24 h, statistically significant (P<0.05). **CONCLUSION** Ouabain induces ECV304 apoptosis by increasing ［Ca^{2+}］$_i$, ROS concentration and up-regulating caspase 3 expression.

Key words ouabain endothelial cells apoptosis calcium reactive oxygen species caspase 3

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