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论著

远志皂苷对β淀粉样蛋白片段1-40诱导PC12细胞凋亡的抑制作用

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摘要 目的 探讨远志皂苷抑制β淀粉样蛋白片段1-40($A\beta_{1-40}$)诱导的PC12细胞凋亡的作用机制。方法 采用聚集状的$A\beta_{1-40}$ 25 μmol·L^{-1}诱导PC12细胞凋亡，然后将处理后的PC12细胞分为$A\beta_{1-40}$模型组和远志皂苷50，100和200 μmol·L^{-1}组，同时设正常细胞对照组。采用MTT比色法检测细胞存活率；膜联蛋白-Ⅴ和PI双染法检测细胞凋亡率；免疫细胞化学法检测细胞凋亡基因Bcl-2和Bax及细胞色素c(Cyt c)表达阳性的细胞百分率；Western印迹法检测PC12细胞中Cyt c的表达水平。结果 与正常对照组比较，$A\beta_{1-40}$模型组PC12细胞的存活率明显降低(P<0.01)，为(31±7)%；Bcl-2阳性表达细胞率降低(P<0.01)，为(23.9±1.9)%；Bax和Cyt c阳性表达细胞率升高(P<0.01)，分别为(79.0±3.7)%和(49.2±3.6)%，Bcl-2/Bax阳性表达细胞比值为0.30。与模型对照组比较，远志皂苷50，100和200 μmol·L^{-1}作用24 h后，细胞存活率分别升高至(51±13)%，(64±7)%和(84±10)%(P<0.01)；Bcl-2阳性率升高至(38.7±0.9)%，(53.7±1.6)%和(60.3±0.8)%(P<0.01)，Bax阳性率分别降低为(60.8±1.9)%，(41.5±2.2)%和(32.7±1.4)%(P<0.01)，Bcl-2/Bax比值亦分别上升为0.64，1.29和1.84；Cyt c阳性率分别降低至(45.4±3.4)%，(30.2±2.2)%和(27.5±1.0)%(P<0.05，P<0.01)。与正常对照组比较，模型组PC12细胞凋亡率和Cyt c蛋白表达水平亦明显升高(P<0.01)；远志皂苷50，100和200 μmol·L^{-1}作用24 h，PC12细胞凋亡率和Cyt c表达水平较模型组均降低(P<0.01)。结论 远志皂苷对$A\beta_{1-40}$诱导的PC12细胞凋亡具有明显的抑制作用，其作用机制可能是抑制Bax和Cyt c表达，增加Bcl-2表达和Bcl-2/Bax比值，从而阻断内源性细胞凋亡通路。

关键词 远志皂苷 β淀粉样蛋白片段1-40 PC12细胞 细胞凋亡 细胞色素c

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Protection of tenuigenin against apoptosis of PC12 cells induced by amyloid beta-protein fragment 1-40

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Abstract

OBJECTIVE To investigate the protective mechanism of tenuigenin (TEN) on the apoptosis of PC12 cells induced by amyloid beta-protein 1-40($A\beta_{1-40}$) *in vitro*. **METHODS** Aggregated $A\beta_{1-40}$ 25 μmol·L^{-1} which induced the apoptosis of PC12 cells was used to establish Alzheimer's disease neuronal cell model. These model neurons were divided into $A\beta_{1-40}$ model group and TEN 50, 100 and 200 μmol·L^{-1} groups. At the same time, normal cell control group was established without $A\beta_{1-40}$ pretreatment. The survival rate of PC12 cells was detected by MTT assay. The apoptosis rate of PC12 cells was detected by flow cytometery with Annexin-Ⅴ/PI double staining. The rates of positive cells expressed Bcl-2, Bax and cytochrome c (Cyt c) were observed by immunocytochemical method. The expression level of Cyt c was detected through Western blotting analysis. **RESULTS** Compared with normal control group, survival rate of PC12 cell decreased to (31±7)%(P<0.01), the positive rate of Bcl-2 was declined to (23.9±1.9)%(P<0.01), the positive rate of Bax and Cyt c increased to (79.0±

3.7)% and (49.2±3.6)% (P<0.01), respectively, and the ratio of Bcl-2/Bax was 0.30 in $A\beta_{1-40}$ model group. Compared with model group, after 24 h incubation of with PC12 cells TEN 50,100 and 200 μmol • L^{-1}, PC12 cell survival rate increased to (51±13)%,(64±7)% and(84±10)%(P<0.01), respectively;the positive rate of Bcl-2 increased to (38.7±0.9)%, (53.7±1.6)% and(60.3±0.8)% (P<0.01),respectively;the positive rate of Bax was declined to (60.8±1.9)%, (41.5±2.2)% and (32.7±1.4)%(P<0.01), and the ratio of Bcl-2/Bax increased to 0.64, 1.29 and 1.84; the positive rate of Cyt c decreased to (45.4±3.4)%, (30.2±2.2)% and (27.5±1.0)%(P<0.05,P<0.01). Compared with normal control group, the apoptosis rate in PC12 cells and expression level of Cyt c protein were also obviously elevated (P<0.01);and after 24 h incubation of TEN 50,100 and 200 μmol • L^{-1} with PC12