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论著

知母皂苷对淀粉样β蛋白片段25~35引起的神经细胞凋亡的保护作用

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摘要 目的 研究知母皂苷（SAaB）对淀粉样β蛋白片段25～35（$A\beta_{25\sim35}$）激活巨噬细胞引起神经细胞凋亡的保护作用及有关的机制。方法 体外培养小鼠腹腔巨噬细胞24 h，加入 $A\beta_{25\sim35}$（20 μmol·L^{-1}），分别在加入$A\beta_{25\sim35}$ 0.5, 1, 2和6 h取巨噬细胞，应用Western印迹方法检测不同时间点的胞外信号调节激酶1/2（ERK1/2）和丝裂原活化蛋白激酶p38（p38MAPK）的蛋白表达改变，确定ERK1/2和p38 MAPK蛋白表达达峰时间。然后，在加入$A\beta_{25\sim35}$前10 min，加入SAaB（10, 30和100 μmol·L^{-1}）或在加入$A\beta_{25\sim35}$前30 min，分别加入p38 MAPK的特异性阻断剂SB203580和ERK上游激酶MEK的特异性阻断剂PD98059，分别在$A\beta_{25\sim35}$作用0.5 和2 h后，取细胞进行Western印迹实验。$A\beta_{25\sim35}$作用48 h后，取培养的巨噬细胞上清液测定肿瘤坏死因子-α（TNF-α）及一氧化氮（NO）生成量的改变，应用免疫细胞化学染色观察巨噬细胞诱导型一氧化氮合酶（iNOS）的表达。为了观察SAaB对$A\beta_{25\sim35}$激活巨噬细胞所介导的神经细胞凋亡的保护作用，在巨噬细胞培养液内加入SAaB（10, 30和100 μmol·L^{-1}）作用10 min，然后加入$A\beta_{25\sim35}$（20 μmol·L^{-1}）作用48 h 后，将培养的上清液转移到体外培养8 d的小脑颗粒细胞内作用72 h，对照组将未被$A\beta_{25\sim35}$刺激的巨噬细胞上清液加入到神经细胞内。应用Hoechst 33258染色观察小脑颗粒细胞凋亡改变。结果 $A\beta_{25\sim35}$（20 μmol·L^{-1}）可使巨噬细胞磷酸化ERK1/2和磷酸化p38 MAPK表达明显增加，分别在加入$A\beta_{25\sim35}$后0.5 h和2 h作用达高峰。另外，$A\beta_{25\sim35}$也可使巨噬细胞的TNF-α和NO产生增加以及iNOS表达增加，$A\beta_{25\sim35}$引起的巨噬细胞TNF-α产生增加是通过ERK1/2信号通路激活介导的，因为MEK的特异性阻断剂PD98059可明显抑制$A\beta_{25\sim35}$引起的巨噬细胞TNF-α产生增加。将 $A\beta_{25\sim35}$刺激48 h的巨噬细胞上清液加入到培养的小脑颗粒细胞内，可使神经细胞凋亡百分比较对照组明显增加。SAaB（30和 100 μmol·L^{-1}）能明显抑制$A\beta_{25\sim35}$引起的巨噬细胞磷酸化ERK1/2、磷酸化p38 MAPK和iNOS表达增加，SAaB （10, 30和100 μmol·L^{-1}）也能对抗$A\beta_{25\sim35}$引起的TNF-α和NO的生成增加及明显降低由$A\beta_{25\sim35}$激活巨噬细胞所介导的神经细胞凋亡。结论 SAaB对$A\beta_{25\sim35}$激活巨噬细胞引起神经细胞凋亡具有对抗作用，该作用与其抑制巨噬细胞的ERK1/2信号转导通路，进而抑制巨噬细胞TNF-α和NO的产生有关。

关键词 皂苷类, 知母 淀粉样β蛋白 MAP激酶信号转导系统 p38 MAP激酶 细胞凋亡
分类号 R966

Saponins from *Anemarrhena asphodeloides* Bge. protect neurons from amyloid β-protein fragment 25-35-induced apoptosis

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Abstract

AIM To investigate the neuroprotective effects and possible mechanisms of saponins from *Anemarrhena asphodeloides* Bge. (SAaB) on neuronal damage induced by amyloid β-protein fragments 25-35 ($A\beta_{25-35}$). **METHODS** Cultured mouse peritoneal macrophages were stimulated with $A\beta_{25-35}$ (20 μmol·L^{-1}) for 0.5, 1, 2 and 6 h or preincubated with SAaB (10, 30 and 100 μmol·L^{-1}) for 10 min or mitogen-activated protein kinase (MAPK) specific inhibitors (p38 MAPK inhibitor SB 203580 and MEK specific inhibitor PD98059) for 30 min prior to the addition of $A\beta_{25-35}$ (20 μmol·L^{-1}). After stimulation with $A\beta_{25-35}$ for the indicated times, total cellular extracts were prepared for Western blotting of extracellular signal-regulated kinase (ERK) and p38 MAPK. After stimulation with $A\beta_{25-35}$ for 48 h, the supernatants of cultured macrophages were collected for quantification of tumor necrosis factor-α (TNF-α) and nitric oxide (NO) and protein expression of inducible nitric oxide synthase (iNOS) in macrophages was determined by immunocytochemical staining. To

determine whether SAaB ?has protective effect against neuronal apoptosis mediated by $A\beta_{25-35}$-induced macrophages activation, macrophages were stimulated with $A\beta_{25-35}$ in the presence or absence of SAaB （10, 30 and 100 μmol·L^{-1}） for 48 h and then the cell-free supernatant of $A\beta_{25-35}$-stimulated macrophages was transferred to the culture of cerebellar granule neurons for 72 h. Neuronal apoptosis was quantitated by scoring the percentage of cells with apoptotic nuclear morphology after Hoechst 33258 staining. **RESULTS** $A\beta_{25-35}$（20 μmol·L^{-1}） significantly induced increase in phosphor-ERK1/2 and phosphor-p38 MAPK protein expression without affecting total protein levels and in the production of TNF-α and NO in cultured macrophages. $A\beta_{25-35}$-induced increase of TNF-α production in macrophages involved activation of ERK1/2 signal pathway. Importantly, TNF-α and NO generated by cultured macrophages after $A\beta_{25-35}$ stimulation may be responsible for the majority of the neuronal apoptosis. SAaB (30 and 100 μmol·L^{-1}) significantly suppressed $A\beta_{25-35}$-induced increase in phosphor-ERK1/2 and phosphor-p38 MAPK protein. In addition, SAaB (10, 30 and 100 μmol·L^{-1}) also decreased the level of TNF-α and NO in supernatants of cultured macrophage and inhibited $A\beta_{25-35}$-induced increase in iNOS protein expression of macrophages. Neuronal apoptosis mediated by $A\beta_{25-35}$-induced macrophage activation was also significantly attenuated by treatment with SAaB (10, 30 and 100 μmol·L^{-1}). **CONCLUSION** SAaB protects neurons against the neuronal cell death induced by $A\beta_{25-35}$. The beneficial effects of SAaB may be related to the reduction of TNF-α and NO from activated macrophage induced by $A\beta_{25-35}$.

Key words saponins Anemarrhena asphodeloides Bge. amyloid beta-protein MAP ?kinase signaling system p38 MAP kinase apoptosis

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