PA-A-33

鐵過多減少發炎反應引起神經微膠質細胞一氧化氮生合成與內質網壓力產生之關係

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已知內質網壓力引起 XBP1 的表現與誘發型一氧化氮合成酶（iNOS）蛋白質的表現有關，而鐵過多會減少發炎反應引起一氧化氮的生成，但其調節機制不詳。因此本實驗欲探討發炎及鐵過多對於神經微膠質 BV2 細胞內質網壓力產生與一氧化氮生成之關係。以 5 μg/ml LPS 與 20 U/ml IFN-γ 處理 24 小時引起發炎反應，其 NO^{2-} 的分泌量增加約 5 倍，同時其 iNOS 蛋白質表現量則顯著增加。將細胞長期培養於 0.2 mM $FeSO_4$ 長達 7 天後，再投予 LPS 與 IFN-γ 處理 24 小時， 其 NO^{2-} 分泌量與 iNOS 蛋白質的表現量均減少分別為 LPS+IFN-γ 處理組之 65%與 61%，表示鐵過多會抑制細胞 NO 的生成。另外，LPS 與 IFN-γ 處理 6 小時可使內質網壓力指標 XBP-1 基因表現顯著達 1.5 倍，並且受其調控之下游基因 CHOP 基因亦隨處理時間而增加表現，而內質網摺疊蛋白 Grp78 蛋白質的表現則不具顯著性差異。然而鐵過多對於發炎反應引起內質網壓力產生的指標不具減少之作用。由此可見發炎反應雖可引起神經微膠質 BV2 細胞中 XBP1 基因的表現，亦可促進一氧化氮的大量生成，但 XBP1 參與 iNOS 基因的分子調控機制尚不足於解釋鐵過多減少發炎反應引起一氧化氮生成之實因。

關鍵詞：鐵過多，發炎，一氧化氮，內質網壓力

PA-A-34

Neuroprotective effect of carnosic acid on 6-hydroxydopamine-induced apoptosis mediated by glutathione synthesis in SH-SY5Y cells

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Understanding the neuroprotective effects of the rosemary phenolic diterpene carnosic acid (CA) has attracted increasing attention. We explored the mechanism by which CA modulates the neurotoxic effects of 6-hydroxydopamine (6-OHDA) in SH-SY5Y cells. Cells were pretreated with CA for 12 h followed by treatment with 100 μM 6-OHDA for 12 or 24 h. Cell viability determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide (MTT) assay indicated that 0.1 to 1 μM CA dose-dependently attenuated the cell death induced by 6-OHDA, whereas the effect of 3-5 μM CA was weaker. CA at 1 μM suppressed the 6-OHDA-induced nuclear condensation, reactive oxygen species generation, and cleavage of caspase 3 and PARP. Immunoblots showed that the phosphorylation of c-Jun NH_2-terminal kinase (JNK) and p38 by 6-OHDA was reduced in the presence of CA. Incubation of cells with CA resulted in significant increases in the total glutathione (GSH) level and the protein expression of the γ-glutamylcysteine ligase catalytic subunit and modifier subunit. L-Buthionine-sulfoximine, an inhibitor of GSH synthesis, attenuated the effect of CA on cell death and apoptosis. Treatment with CA also led to an increase in nuclear factor erythroid-2 related factor 2 (Nrf2) activation, antioxidant response element (ARE)-luciferase reporter activity, and DNA binding to the ARE. In conclusion, the data suggest that CA attenuates 6-OHDA-induced apoptosis through down-regulation of the JNK and p38 signaling pathways in SH-SY5Y cells. Moreover, this protective effect is associated with the induction of the GSH synthesis system. Our results suggest CA as a potential candidate for protection against neurodegeneration in Parkinson's disease.

Keywords: carnosic acid, glutathione, c-Jun NH_2-terminal kinase, p38 kinase, SH-SY5Y cells