

论文

氨甲酰基促红素的制备及其对过氧化氢损伤的SH-SY5Y细胞保护作用的观察

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摘要:

目的 制备氨甲酰基促红细胞生成素并研究其对H₂O₂损伤的SH-SY5Y细胞的保护作用。**方法** 将促红细胞生成素与氰酸钾共同作用使其转化为氨甲酰基促红细胞生成素, 并通过电泳鉴定。分别选用300、400μmol/L H₂O₂作用于SH-SY5Y细胞12h后分别加入相同浓度的氨甲酰基促红细胞生成素及促红细胞生成素, 作用24h终止培养, 分别测定各组的噻唑兰(MTT)、乳酸脱氢酶值, 通过Annexin V /PI双染色后经流式细胞仪检测细胞凋亡率, 采用RT-PCR法检测Bcl-2及Caspase-8的表达, 比较氨甲酰基促红素及促红素对H₂O₂损伤的SH-SY5Y细胞的保护作用, 并探讨其作用机制。**结果** 成功将促红素已氨甲酰化为氨甲酰基促红素。经氨甲酰基促红细胞生成素与促红细胞生成素作用后, H₂O₂损伤的SH-SY5Y细胞的MTT值增高, 乳酸脱氢酶降低, 细胞凋亡率减少。**结论** 氨甲酰化促红素对氧自由基损害的SH-SY5Y细胞具有与促红素同样的保护作用, 且在一定浓度范围内保护程度随着浓度的升高而增加。

关键词: 氨甲酰基促红素; 促红素; 过氧化氢; SH SY5Y细胞; 细胞凋亡

Preparation of carbamylated erythropoietin and its protective effect on SH-SY5Y cells injured by hydrogen peroxide

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Abstract:

Objective To prepare carbamylated erythropoietin(CEPO), and to investigate protective effects and possible mechanisms of CEPO and EPO on SH-SY5Y cells injured by hydrogen peroxide(H₂O₂).
Methods After EPO was carbamylated by reaction with potassium cyanate, CEPO was prepared and identified by using sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE), which could not be digested by endoproteinase Lys-C. In order to observe their protective effects against oxygen-derived free radical damage, different concentrations of CEPO and EPO were added to the SH-SY5Y cell culture after exposure to 300 and 400μmol/L of H₂O₂ for 12 hours. Then living cellswere measured by 3-(4,5)-dimethylthiaziazolo (-z-y1)-3,5-di-phenyltetrazoliumromide(MTT) and lactate dehydrogenase, apoptosis was measured by Annexin V/PI immunofluorescent staining and laser scanning confocal microscopy, and Bcl-2 and Caspase-8 were tested by RT-PCR. **Results** The electrophoretic pattern showed that EPO was successfully prepared and had a high purity. Both EPO and CEPO supplementation groups displayed a significant increase in MTT and a decrease in LDH. Also, there were striking decreases in apoptosis and expression of Caspase-8 and an increase in expression of Bcl-2. There was no difference between the effects of EPO and CEPO. When concentrations of EPO and CEPO reached 100IU/mL, the dose-dependent protection of EPO was maximized. **Conclusion** Both CEPO and EPO can evidently protect SH-SY5Y cells against oxygen-derived free radical damage, and there is no difference between them. The dose-dependent protective effects of CEPO and EPO are increased with the concentration.

Keywords: Carbamylated erythropoietin; Erythropoietin; Hydrogen peroxide; SH-SY5Y cells; Apoptosis

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