甲基乙二醛對粒線體蛋白質之碳醯化修飾作用

Methylglyoxal-modified carbonyl proteins in mitochondrial

中文摘要

甲基乙二醛（Methylglyoxal，MGO）會和蛋白質以共價鍵鍵結而形成高度醣化終產物(Advanced Glycation Endproducts, AGEs)，AGEs 累積會使得蛋白質結構改變與失去正常功能，進而引起某些生理疾病，包括糖尿病與阿茲海默症。先前有研究指出，甲基乙二醛會造成腎臟環間膜細胞（glomerular mesangial cells）細胞凋亡（apoptosis），本篇研究中，我們首先嘗試以丹參酮 IIA（tanshinone IIA）抑制甲基乙二醛對大鼠腎臟環間膜細胞所造成之細胞毒害（cytotoxic），實驗結果顯示丹參酮 IIA 對大鼠腎臟環間膜細胞有些微細胞保護作用（cytoprotective effect）。接著我們利用 DNPH（2,4-dinitrophenylhydrazine）衍生物之免疫法分析甲基乙二醛對蛋白質之碳醯化（carbonylation）程度，並藉由蛋白質二維電泳進一步分析。目前已知過度醣化會造成粒線體的功能喪失而引發糖尿病併發症，因此我們進一步以飛行質譜儀分析被甲基乙二醛碳醯化之大鼠大腦粒線體蛋白質的身分，經由飛行質譜儀分析出 pyruvate kinase M、dihydropyrimidinase-related protein 2、heat shock protein 70、beta/gamma actin 及 alpha tubulin 會受到甲基乙二醛碳醯化。由於這些蛋白質都和許多神經退化相關疾病有關，很可能在過度糖化所引起粒線體功能喪失之糖尿病併發症的致病機制中扮演重要角色。

英文摘要

Methylglyoxal (MGO) binds covalently to proteins resulting in the formation of advanced glycation end products (AGEs). AGEs can alter protein structure and functions and involve in several pathological processes, including diabetes mellitus (DM) and Alzheimer’s disease (AD). Previous studies have indicated that MGO can induce cell apoptosis in glomerular mesangial cells. In the present study, we first investigated whether MGO induces cytotoxic effects in rat glomerular mesangial cells and whether tanshinone IIA exerts a cytoprotective effect against MGO-induced cytotoxicity. Our results revealed that tanshinone IIA exerts little cytoprotective effect in glomerular mesangial cells. We next examined whether treatment of mesangial cells with MGO results in protein carbonylation using DNPH-derived immunoblot assay. Incubation of mesangial cells with MGO resulted in significant increase of protein carbonylation as revealed by two-dimensional electrophoresis (2D). Because mitochondrial dysfunction is the hallmark of diabetic complications, we then characterized the carbonylated proteins in isolated rat brain mitochondria using matrix-assisted laser desorption/ ionization mass spectrometry (MALDI-TOF). We demonstrated that pyruvate kinase muscle isozyme (PKM),

dihydropyrimidinase-related protein 2 (DRP-2), heat shock protein 70 (Hsp70),beta/gamma actin and alpha tubulin were carbonylated by MGO. Given modification of these mitochondrial proteins have been linked to a variety of neurodegenerative diseases, these findings may be important in understanding the mechanisms by which hyperglycemia induces mitochondrial dysfunction in diabetic complications.