過度糖化最終產物在糖尿病併發症中所扮演的角色

Roles of Advanced Glycosylation End Products (AGEs) in Diabetic Complication

中文摘要

1) 評估過度糖化最終產物在糖尿病病人血清中的含量還原糖與蛋白質的氨基端反應之後 ，形成各種不同之具有螢光特質之過度糖化最終產物(Advanced Glycasylation end products, AGEs)。血清中之低分子量之 AGEs(Low Molecular Weight-AGEs, LMW-AGEs)已被證實與糖尿病之心臟血管方面的併發症有關 ，在吾人之研究中 ，已成功的從動物體內獲得對抗 AGEs 之專一性抗體 ，且建立了一套檢測糖尿病病人血清中之 LMW-AGEs 的競爭型酵素免疫吸附法(Enzyme-liked immunosorbent assay, ELISA) , 用以檢測非糖尿病患者之年輕族群與老人族群及患有糖尿病之病人血清中 LMW-AGEs 的含量。吾人將競爭型 ELISA 實驗中 , 5 倍稀釋之混合血清(pooled serum)獲得之 B/Bo 的數值定義爲一個 AGE unit (AU) , 並且發現在糖尿病病人血清中 AGEs 的含量明顯地(8.96±2.13 AU, N=32)較年輕族群(3.12±0.52 AU, N=30)和老人族群(4.41±1.1AU, N=36)高($p<0.05$)。吾人實驗中獲得之糖尿病病人之血清中 AGEs 的含量與病人先前所檢測之 HbA1c 的結果在統計學上具有相關性(R=0.86)。從吾人實驗的結果可以明確地了解糖尿病病人血清中 AGEs 的含量確實較正常人高 ，而吾人認爲檢測糖尿病病人血清中 AGEs 的含量 ，將可作爲糖尿病併發症之指標。

(2)過度糖化最終產物在 C6 神經膠瘤細胞中引發 Cox-2 表現之作用過度糖化最終產物(Advanced glycosylation end products, AGEs)是糖類和蛋白質經由非酵素性作用的衍生物，當糖尿病病人或老年人長期處於高血糖(hyperglycemia)狀態時，體內這些無法代謝完全之糖類就容易和一些長半衰期的蛋白質反應形成 AGEs。在吾人之研究中發現 C6 神經膠瘤細胞(glioma cells)加入 BSA-AGEs 處理 24 小時後，可引發性之 Cyclooxygenase-2 (COX-2)的表現有被引發(induced)的現象，且加以 arachidonic acid 之後，prostaglandin E2(PGE2)的含量也明顯的增加。若以 p38 MAP kinase 的抑制劑-SB203580 和 NF-kB 的抑制劑-PDTC 事先處理細胞，發現 BSA-AGEs 刺激 C6 glioma cells 所引發之 COX-2 的表現或是 PGE2 的產量都有明顯的減少。因此，由吾人之結果判斷 BSA-AGEs 所造成之 COX-2 的表現或是 PGEs 的產生，其訊號傳遞(signal transduction)的路徑應該是經由 MAP kinase/ NF-kB 之路徑。故此一路徑可能扮演著 AGEs 在糖尿病病患所造成之神經病變時重要的角色。

(3)過度糖化最終產物在 C6 神經膠瘤細胞中引發 iNOS 之表現過度糖化最終產物(Advanced glycosylation end products, AGEs)在不同的細胞株(cell-line)中已經被發現可以引發 iNOS 的表現，在吾人的實驗中同樣地也發現 AGEs 可以刺激 C6 glioma cell 表現 iNOS。加入抗 AGEs 之抗體處理細胞之後，發現 AGEs 在 C6

glioma cell 中引發出的 iNOS 之活性明顯地被阻斷。因為 AGEs 在細胞內觸動的 p21ras 訊息傳遞與 AGEs 所造成之細胞內氧化壓力(oxidant stress)有關。為了進一步確認此一事件，吾人利用 l-Buthionine-(S,R)-Sulfoximine(一種可抑制細胞內之 glutathion 還原能力的抑制劑)來加強 AGEs 在細胞內所產生的氧化壓力，結果發現 NO 的產量如預期的增加了一倍以上。相反地，若以 L-NAC 來抵抗此氧化壓力，結果 NO 的產量會受到抑制。PD98059 (MAPKK 的抑制劑)、SB203580 (p38 MAPK 的抑制劑)和 PDTC (NF-kB 的抑制劑)在吾人的實驗中都有效地抑制了 AGEs 所刺激之 iNOS 的表現，因此 AGEs 刺激 C6 glioma cells 表現 iNOS 的訊息傳遞路徑中，可能經過了 MAPK/NF-kB 的路徑。加入 l-NAME(非選擇性之 NO 合成酵素之抑制劑)處理細胞之後，發現 iNOS 的活性被受抑制，因此可以證明在 C6 glioma cells 中產生之 nitrite 實為 iNOS 的活性所致。在另外的實驗中，吾人發現 Go6976(PKC 的抑制劑)對於 NO 的產生也有明顯的抑制效果，因此 AGEs 在 C6 glioma cell 中引發 iNOS 表現的訊息傳遞路徑可能也經過了 PKC 的傳遞路徑。綜合以上的結果，吾人認為 MAPK/NF-kB 的路徑在 AGEs 引發 iNOS 的訊息傳遞中扮演著很重要的媒介。PKC 在此訊息傳遞路徑中扮演著何種角色與 AGEs 藉由何種作用機制活化 PKC，則需要日後的實驗作進一步的證明。

英文摘要

(1) Reducing sugars react with amino groups of proteins to form a variety of fluorescent advanced glycosylation end products (AGEs). Serum low molecular weight-AGEs (LMW-AGEs) have been linked to the development of diabetic-associated cardiac vascular complications. In this report, we raised antibodies specifically against AGEs and developed a competitive enzyme-linked immunosorbent assay (ELISA) to determine the serum LMW-AGEs in two age groups of non-diabetics and one group of diabetics. By defining 1 AGEs unit (AU) as the inhibition that results from 1:5 diluted pooled serum in competitive ELISA, We found that the circulatory AGEs levels in the young group as well as those in the elderly group of non-diabetics fit normal distributions ($P<0.05$) and their reference ranges were 3.12 0.52(N=30) and 4.41 1.2 AU (N=36), respectively. The circulatory AGEs levels in diabetic patients were 8.96 2.13 AU (N=32) which is significantly higher than that in both age groups of non-diabetics ($P<0.01$). The circulation AGEs data correlated well with the HbA1c values obtained from patients with diabetes ($r=0.86$). In conclusion, these data reveal that circulation AGEs are higher in the elderly as compared to those of the young group and may serve as a circulation marker reflecting the severity of the diabetic sequel.

(2) AGEs are the reactive derivatives of non-enzymatic glucose-macromolecules condensation products. These compounds have been implicated in the structural and functional alterations of proteins that occur during aging and long-term diabetes. In

the present studies, rat C6 glioma cells were incubated with the BSA-AGEs, the Cox-2 protein expression and PGE2 production were examined. Since the MAPKK inhibitor, PD98059,the p38 MAP kinase inhibitor, SB203580 and the NF-kB inhibitor, PDTC abolished the BSA-AGEs-induced COX-2 expression and PGE2 production, MAP kinase and NF-kB appear to be involved in the signaling pathway. Our data suggest that the AGEs-induced COX-2 expression and PGE2 production are mediated through MAP kinase/ NF-kB pathways which may play a role in the progression of neurological disorders implicated with diabetic mellitus.
(3)AGEs stimulated a dose dependent NO production from a variety of cell lines. In the present study, we demonstrated that AGEs stimulated inducible NO synthase (iNOS) expression as well as NO production in C6 glioma cell line. The AGEs-stimulated NO production was blocked by anti-AGEs Ab in C6 glioma cells. The AGEs—stimulated NO production from C6 glioma cell was inhibited by PD98059, the MAPKK inhibitor, SB203580 (10uM), the p38 MAPK inhibitor and PDTC (50uM), the NF-kB inhibitor. Thus, AGEs-induced iNOS expression may mediate through the MAPK/NF-kB pathway. The NO synthase non-selective inhibitor, l-NAME inhibited the AGEs-stimulated NO accumulation suggesting that the NO accumulation may be due to iNOS expression. Since the AGEs-stimulated p21ras activation is mediated by increasing intracellular oxidative stress, we investigate the role of antioxidant, l-NAC on AGEs-stimulated NO release from C6 glioma cells. Consistently, the endogenous glutathione inhibitor, l-Buthionine-(S,R)-Sulfoximine, enhanced AGEs-stimulated NO production in C6 glioma cells. Pretreatment of C6 glioma cells with PKC inhibitor, Go6976, specifically inhibited the subseqeunt AGEs-stimulated NO release from C6 glioma cells, suggesting that PKC may also involve in AGEs-stimulated NO production. In conclusion, although the detail signaling mechanism is not totally understood, the AGEs-stimulated effect is clearly dependent on MAPK/NF-kB pathway mediated by oxidative stress. Possibly AGEs also trigger the PKC pathway in iNOS expression.