# 過度糖化最終產物之臨床實驗應用

# Application of Advanced Glycosylation End Products in Clinical Laboratory

## 中文摘要

醣類之羰基與蛋白質之胺基經由梅納反應產生非酵素性之共價結合,進而形成一系列棕色而帶有螢光的產物,通稱爲過度糖化最終產物(Advanced Glycosylation End Products).近十年來廣泛的研究顯示,此種不可復原性之產物在糖尿病併發症如腎臟病變,視網膜病變,白內障,糖尿病足,心血管疾病,動脈粥狀硬化,甚至阿茲罕默氏症(Alzheimer disease)中皆扮演著舉足輕重的角色,當然其與老年人心血管疾病之好發亦有著極大的相關性存在.本研究藉藉由體外試驗的方式,將蛋白質與各種不同之醣類相互作用,以明瞭此不同醣類之間形成過度糖化最終產物之差異性;同時利用所合成之物質作爲抗原,用以製造抗AGEPs之抗體,並以此抗體作爲臨床檢驗之工具,用以篩檢糖尿病病人及老年人體內AGEPs之分佈情形,並研究發展出一臨床檢驗法以監控糖尿病及老年人體內AGEPs之累積情形.而在這整個實驗中發現,五碳糖形成過度糖化最終產物之速率較六碳糖快了許多,且其螢光性約爲六碳糖之十倍左右,而在經由數週之培養後,其會發生相當嚴重之交叉連結(Cross-linking),並且在電泳結果上觀察到Bend shift的現象,由此結果可使吾人對於糖尿病人飲食中六碳糖取代物之選用重新地加以評估;另外利用免疫組織化學染色法(Immunohistochemistry)及酵素結合式免疫吸附分析法(Enzyme Linked Immunosorbent Assay簡稱ELISA),我們發現在一些糖尿病人之組織切片中,會有高量之AGEPs沉澱其中,此類物質很可能是因長期性高血糖(Hyperglycemia)與體內一些長半衰期之蛋白質(Long-lived protein)如collagen相互作用所形成,其沉積於體內血管壁與組織之中,會造成體內血管阻塞因而引發一系列之併發症產生,而糖尿病人之LDL亦同樣會有過度糖化之情形發生.由此可知過度糖化最終產物實與糖尿病併發症有著密不可分之關聯性存在,其主要原因可能是此種物質在體內會引發某種程度之免疫反應,導致自體免疫系統遭受破壞所致,此則有待更進一步實驗之證實;至於我們所發展之臨床免疫檢驗法則不但可用來檢驗患部的組織中AGEPs的累積情形,也可用來檢驗血清中LDL-AGEPs的濃度,後者不但可做爲非侵襲性之循環指標,還可使我們更快地篩檢出危險性高之族群,並對其飲食及各方面加以控制,避免其併發症之產生,以達預防勝於治療的目的.

## 英文摘要

Reducing sugars can react nonenzymatically with free amino

groups on proteins and form a variety of fluorescence-producing advanced glycosylation end products (AGEPs). These irreversible compounds have previously identified to accumulate on long-lived extracellular matrix proteins and probably also onDNA in tissues that develop diabetic complications. In this study, we examinedthe rates of variable carbohydrates including glucose, galactose, mannose, fructose, arabinose and xylose in stimulating AGEPs by incubating these sugarswith bovine serum album in in vitro. The accumulations of AGEPs were be monitored by fluorescence detection at 410 nm . After 1 week incubation, arabinose and xylose stimulate 10 folds more AGEPs than other sugars. Further incubation,protein cross-linking were be found in these two sugars resulted in shift ofelectrophoretic motility. Fructose elicited 150% more AGEPs than glucose after2 or 3 week's incubation and the differences between fructose and glucosedramatically increased thereafter. In conventional glycemic control, pentoseand fructose have been preferentially employed due to their low absorption rateand greater clearance rate than those of glucose. However, long term usage ofarabinose, xylose and fructose as diet sugar substitutes may have adverse effectsbecause they stimulate more AGEPs accumulation. We also raised the polyclonalantibodies directed against protein-bound AGEPs and developed an immunohistochemical staining to screen the available tissue paraffin blocksfrom patients with or without diabetes. Our results demonstrate that AGEPs accumulate in many diabetics' tissues. We also used Enzyme Linked ImmunosorbentAssay (ELISA) to detect the amount of LDL-AGEPs in diabetes and elder.These results clearly show that high levelofAGEPs products in the two groups than the healthy group. In conclusion, these antibodies can use to detect AGEPs in tissues and serve as a new marker of cardiac vascular complications in diabetes and aging individuals.