行政院國家科學委員會補助專題研究計畫成果報告

過度糖化最終產物對胰島素訊息傳遞的影響

計畫類別：∨個別型計畫　□整合型計畫

計畫編號：NSC91－91－2314－B－038－025

執行期間：　91 年　8 月　1 日至　92 年　7 月　31 日

計畫主持人：李宏謨

共同主持人：

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：　台北醫學大學醫技系

中　華　民　國　92 年　4 月　11 日

行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC 91-2314-B-038-025
執行期限：91 年 8 月 1 日至 92 年 7 月 31 日
主持人： 李宏謨
執行機構及單位名稱台北醫學大學醫技系

一、中文摘要

"過度糖化最終產物" (Advanced Glycosylation End Products，簡稱為AGEs)。已被證實可影響細胞內的訊息傳遞並且是糖尿病併發症的主要病因。因為第二型糖尿病常見於AGEs開始堆積的中老年人，我們假設AGEs可以影響胰島素所刺激的PI 3-kinase之訊息傳遞路徑，進而影響葡萄糖的運送並形成第二型糖尿病。本研究以分化之3T3-L1脂肪細胞，研究AGEs對胰島素所引發的PI 3-kinase之訊息傳遞路徑和葡萄糖運送的影響。以BSA-AGEs(300μg/ml)處理分化過之3T3-L1脂肪細胞24小時後，可發現胰島素所刺激的[^{3}H]2-去氧葡萄糖之運送受到抑制。以AGEs處理3T3-L1脂肪細胞二小時後IRS-1出現doublet，同時大幅誘導IRS-2的蛋白表現。由於AGEs亦可增加PPAR-γ的表現，而PPAR-γ的活化亦可以增加IRS-2的蛋白表現，我們推測IRS-2的upregulation可能導因於AGEs誘導PPAR-γ的表現所致。因為AGEs構造複雜，為了進一步了解AGEs對PI 3-kinase相關的訊息傳遞路徑的影響，我們製備了ε-carboxyl-methyllysine (CML)，並證實 CML亦可抑制胰島素所刺激的 [^{3}H]2-去氧葡萄糖之運送約40%。以CML處理3T3-L1細胞二十四小時後，可以發現Akt/PKB的磷酸化和Akt/PKB的酵素活性均有明顯的減低。因為對胰島素敏感的葡萄糖輸送者(insulin-sensitive glucose transporter)，GLUT-4，即在Akt/PKB的下游；很可能 AGEs是透過抑制Akt/PKB的活性而影響glucose transporter 的表現及轉位，並進一步影響 [^{3}H]2-去氧葡萄糖之運送。為了進一步了解Akt/PKB在AGEs之抑制作用中所扮演的角色，我們製備了過度表現Akt/PKB及kinase dead的Akt/PKB突變基因，並且已成功的送入細胞中，證實Akt/PKB的蛋白表現和磷酸化情形都能依照預期的增減。我們正進一步研究過度表現Akt/PKB的3T3-L1細胞是否能逆轉AGEs的作用。我們目前的結論是AGEs可以透過調節IRS-1及IRS-2的訊息傳遞來影響PI 3-kinase的訊息傳遞，進而影響[^{3}H]2-去氧葡萄糖之運送，過度表現Akt/PKB及kinase dead的Akt/PKB細胞株將有助於了解其詳細的作用機轉。

關鍵詞：AGEs, CML, 胰島素, 訊息傳遞, Akt/PKB

Abstract

Formation of advanced glycation end products (AGEs) is considered a potential link between hyperglycemia and chronic diabetic complications, including disturbances in cell signaling. It was hypothesized that AGEs alter insulin-mediated signaling by interfering with PI 3-kinase dependent pathway. Therefore, we studied

the effects of AGEs on the insulin-stimulated PI 3-kinase dependent signaling transduction pathway in differentiated NIH-3T3 L1 adipocytes. Treatment of 3T3-L1 adipocytes with AGEs inhibited the insulin-stimulated [^{3}H] 2-deoxyglucose uptake in a time- and dose-dependent manner. Inhibition of glucose disposal by AGEs is associated with appearance of IRS-1 doublets presumably due to increased Ser^{307} phsophorylation and an increased induction of IRS-2 expression. Incubation of 3T3-L1 adipocytes with AGEs increased PPAR-γ protein expression suggesting AGEs-induced PPAR-γ expression may affect IRS-2 expression. Furthermore, incubation of 3T3-L1 adipocytes with AGEs reduced Akt/PKB phsophorylation and activation. Given the insulin-sensitive glucose transporter, GLUT-4, is a down stream effector of Akt/PKB, it is possible that AGEs impair the insulin- stimulated glucose transport by inhibiting Akt/PKB activation. In order to demonstrate that Akt/PKB is involved, we have constructed the Akt/PKB overexpression and dominant negative mutant. Transfection of the wild type Akt or overexpression mutants effectively increased the Akt/PKB protein expression and phosphorylation, whereas introduction of the kinase dead mutant inhibits the insulin-induced Akt/PKB pshophorylation. These mutants will prove useful in delineate the mechanism for impaired cellular signal transduction in situations that are associated with diabetes. In conclusion, our data revealed that IRS-1 and IRS-2 undergo differential regulation in 3T3-L1 adipocytes by AGEs. Further works using Akt/PKB overexpression and dominant negative cells will be required to delineate the underneath mechanisms.

Keywords: AGEs, CML, Inuslin, signal transduction, Akt.

二、緣由與目的

Aging or prolonged elevation of glucose levels in diabetic patients results in a number of complications including nephropathy, arteriosclerosis, retinopathy, neuropathy, and cataracts. These complications have been related to advanced glycosylation end products (AGEs). AGEs are fluorescent substances formed by the non-enzymatic "Maillard reaction", and have been considered to be an important factor in mediating diabetic sequelae (Brownlee, 1991). We have previously BSA –AGEs induced iNOS expression and COX-2 expression in many different cell lines. Recently, our research interests focused on elucidating the roles of AGEs in mediating insulin resistant.

Insulin resistance contributes importantly to the pathophysiology of type 2 diabetes mellitus. Although a full understanding of insulin action is evolving, the discovery of insulin receptor substrate (IRS) proteins and their link to the intracellular signaling cascades provided an important step forward. Inhibition of IRS protein functions by serine phosphorylation represents a common molecular mechanism for

insulin resistance. Serine phosphorylation of IRS-1 may lead to impairment in the ability of IRS-1 to activate downstream phosphatidylinositol 3-kinase-dependent pathways. On the other hand, long-term exposure to high glucose increases IRS-2 content in rat adipocytes and it impairs glucose transport capacity together with an impaired sensitivity to insulin stimulation of PKB activity (Bjornholm et al., 2002). In the present study, we investigate the effects of AGEs on insulin-stimulated glucose transport as well as the insulin-mediated signal transduction pathway. We found that treatment of 3T3-L1 cells with AGEs results in impaired glucose transport, which is associated with differential regulation of IRS-1 and IRS-2 and an inhibition of Akt/PKB.

三、結果與討論

Fig. 1. Effects of CML on insulin-stimulated [^{3}H] 2-deoxyglucose uptake in 3T3-L1 adipocytes.

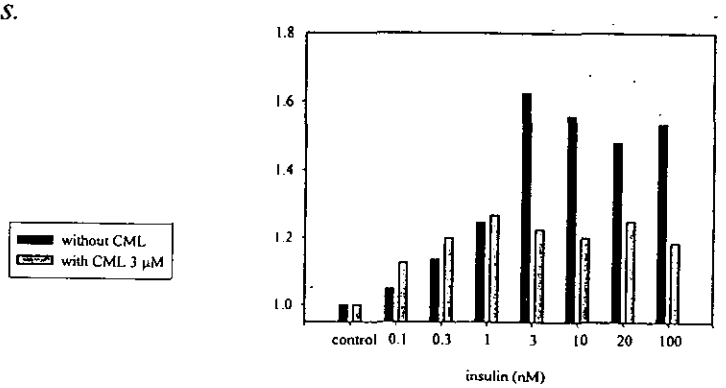


Fig. 2. Effects of BSA-AGEs on IRS-1and IRS-2 expression in 3T3-L1 cells

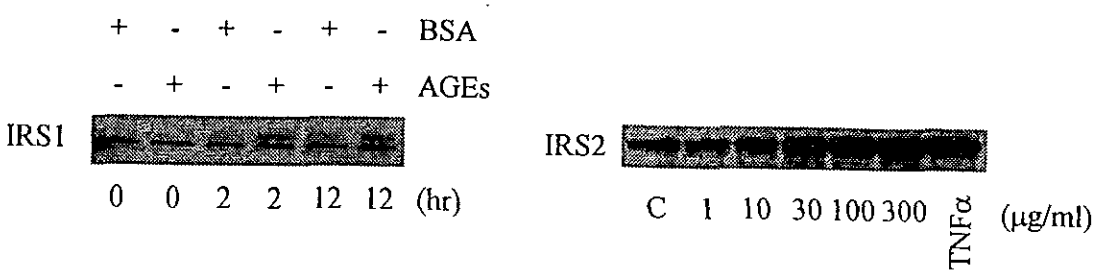


Fig. 3. BSA-AGEs induce the increase in PPAR-γ expression in 3T3-L1 adipocytes.

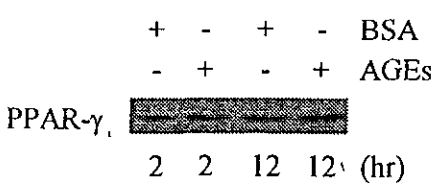


Fig. 4. Effects of ε-carboxyl-methyllysine (CML) on Akt/PKB activation and Akt/PKB kinase activity.

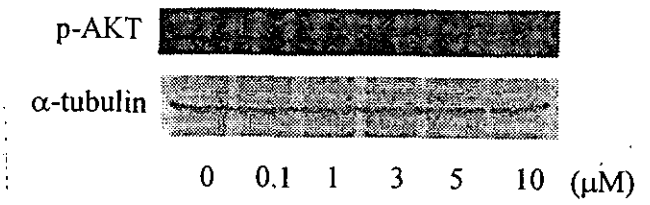
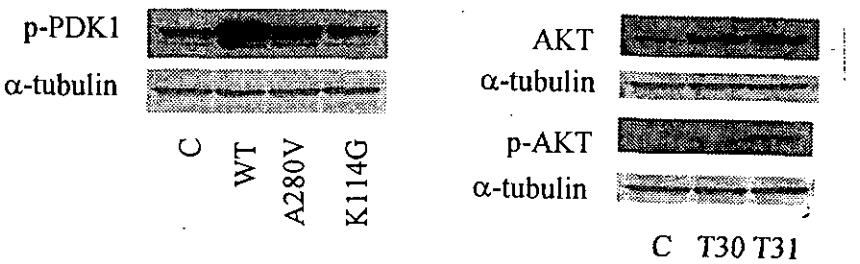


Fig. 5. Expression and phosphorylation of Akt/PKB in cells expression PDK-1 and Akt/PKB mutants.



四、計畫成果自評

我們以過度糖化最終產物研究糖尿病併發症的細胞及分子機轉。我們發現 AGEs 可以又發 iNOS 及 COX-2 的表現這些發現分別發表於 Life Science (69): 2503-2515 **(SCI)**。及 European Journal of Pharmacology 438(3): 143-52. (2002) **(SCI)**。PI-3 Kinase、NFkB 的研究結果則也已發表於 Molecular Cellular Endocrionology (194) 9-17 (2002) **(SCI)**。另外有關 PI-PLC, PC-PLC、PKC 亞型的研究，已投稿 Biochemical Pharmacology (In press) (2003) **(SCI)**；除此之外，我們也研發出的血中 AGEs 自動分析法投稿到 Journal of Clinical Biochemistry *35, (3): 189-195* (2002) **(SCI)**。所以過去一年多我們已發表五篇文章於 SCI 期刊，除此之外，我們還有三篇文章已經投稿，其中一篇投稿於腎臟醫學第二名的 Kidney International 審查意見甚佳，且已經 revise 完成，近期應可被接受。

五、參考文獻

Bjornholm M. Al-Khalili L. Dicker A. Naslund E. Rossner S. Zierath JR. and Arner P. (2002) Insulin signal transduction and glucose transport in human adipocytes: effects of obesity and low calorie diet. *Diabetologia. 45(8):1128-35.*

Smith U. Gogg S. Johansson A. Olausson T. Rotter V. Svalstedt B. (2001) Thiazolidinediones (PPAR-γ agonists) but not PPAR-αagonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes. *FASEB J. 15(1):215- 220.*

White MF, (2002) IRS proteins and the common path to diabetes. Am J Physiol. 283(3):E413-22,