行政院國家科學委員會補助專題研究計畫成果報告

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※　　　　糖尿病併發症的細胞及分子基礎　　　　　　※
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計畫主持人：李宏謨/台北醫學大學醫事技術學系

執行單位：台北醫學大學醫事技術學系

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一、中文摘要

還原糖和蛋白質的氨基(amino group)會發生一種非酵素的Maillard 反應而產生一系列的螢光產物，稱爲"過度糖化最終產物" (Advanced Glycosylation End Products)，簡稱爲AGEs。這些構造非常多樣化的螢光化合物，已被證實能改變組織蛋白的結構和功能。因此糖類雖然是營養物質，但若因組織蛋白過度糖化，卻是糖尿病併發症的主要病因。爲了進一步了解AGEs的致病機制，我們以BSA-AGEs處理C6-glioma細胞，結果發現BSA- AGEs加入後誘導型一氧化氮合成胺(inducible-Nitric Oxide Synthase，簡稱iNOS)的表現明顯增加，其訊息傳遞機制和酪氨酸的磷酸化、Ras和p38MAPK的活化有關，而與MEK及ERK-1/-2等無關。此一發現已發表於Life Science (69): 2503-2515, 2001。我們也發現Raw264.7巨噬細胞中AGEs可以誘導Cyclooxygenase-2 (簡稱COX-2)基因表現，此一發現已被European Journal of Pharmacology 所接受(In press)，我們也探討AGEs所刺激產生的 iNOS表現和Cox-2表現中PI-PLC, PC-PLC、PI-3 Kinase、PKC亞型、NFkB等訊息傳遞物可能扮演的角色。其中有關 PI-PLC, PC-PLC、PKC亞型的研究，已投稿Biochemical Pharmacology ; PI-3 Kinase 、 NFkB 的研究結果則也已投稿 British Journal of Pharmacology。除此之外，我們也將過去研發出來的血中AGEs自動分析法投稿到Journal of Clinical Biochemistry。所以今年我們以投稿五篇文章再SCI 期刊，其中二篇已接受，另三篇正在進行，近期會有消息。本報告僅摘錄其中一篇PLL-AGEs誘導COX-2基因表現的機轉，(將發表於European Journal of Pharmacology)：我們發現在RAW 264.7巨噬細胞中AGEs可以誘導COX-2的表現。此一效應可以被蛋白質tyrosine磷酸化的抑制物，genistein及p38 mitogen-activated protein kinase (MAPK) 的抑制物 SB 203580 所抑制而Ras inhibitor, FPT inhibitor II, 及 MEK inhibitor, PD 98059並無作用，顯示AGEs誘導COX-2的表現是經過tyrosine kinase 及p38MAP kinase的路徑。 因爲AGEs被證實可以活化p38 MAPK的活性，而此一活化作用可被genistein 及 SB 203580所抑制；所以期訊息傳遞的機制是經過tyrosine kinase 及p38MAP kinase的路徑。

關鍵詞：過度糖化最終產物、p38 MAPK、RAW 264.7巨噬細胞、環氧酶-2

Abstract

In the present study, murine RAW 264.7 macrophages were incubated with poly-L-lysine derived - advanced glycosylation end products (PLL-AGEs) to examine cyclooxygenase-2 protein expression. Treatment of RAW 264.7 cells with PLL-AGEs caused a dose-dependent cylooxygenase-2 but not cylooxygenase-1 expression and an increase in COX activity. Increased cylooxygenase-2 expression was seen at 6 h and reached a maximum at 24 h. The tyrosine kinase inhibitor, genistein, and the p38 mitogen-activated protein kinase (MAPK) inhibitor, SB 203580, inhibited PLL-AGEs-induced cylooxygenase-2 expression, while the Ras inhibitor, FPT inhibitor II, and the MEK inhibitor, PD 98059, had no effect on PLL-AGEs-induced cylooxygenase-2 expression. Incubation of RAW 264.7 cells with

PLL-AGEs resulted in activation of p38 MAPK, and this activation was suppressed by genistein and SB 203580. Taken together, our results suggest that activation of protein tyrosine kinase and p38 MAPK is involved in AGEs-induced cyclooxygenase-2 expression in RAW 264.7 macrophages.

Keywords: Advanced glycosylation end products; Cyclooxygenase-2; p38 Mitogen-activated protein kinase; RAW 264.7 cells.

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).

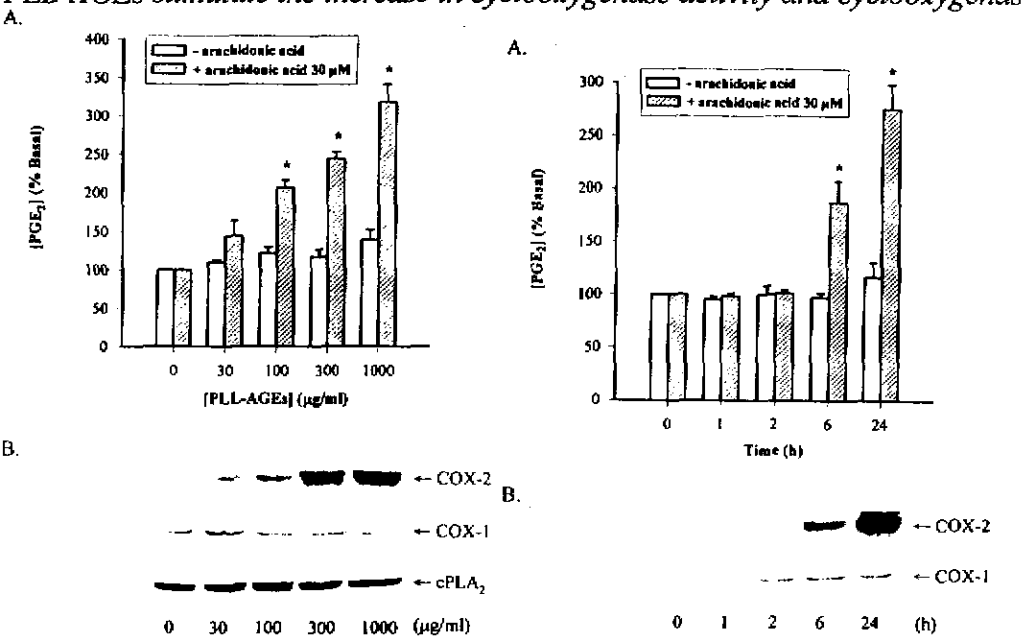
Cyclooxygenase, also known as prostaglandin endoperoxide H synthase, catalyzes the conversion of arachidonic acid to prostaglandin H_2, the precursor of prostanoids. Expression of cyclooxygenase has recently emerged as an important determinant of the cytotoxicity associated with inflammation (Seibert et al., 1995). Cyclooxygenase has two isoforms designated cyclooxygenase-1 and cyclooxygenase-2. Cyclooxygenase-1 is constitutively expressed in many cell types. Cyclooxygenase-2 bears 60% overall identity with cyclooxygenase-1 at the amino acid sequence level.

The signaling pathways of cyclooxygenase-2 gene expression have been studied in a number of cell systems. The expression of cyclooxygenase-2 seems to be closely related with the activation of mitogen-activated protein kinases (MAPKs). There are three important groups of MAPKs, including p44/42 MAPK, also known as extracellular signal-regulated kinase 1/2 (ERK 1/2), stress-activated protein kinase (SAPK)/c-jun N-terminal kinase (JNK), and p38 MAPK. The p44/42 MAPK pathway is preferentially activated by growth factors and mitogens, whereas the SAPK/JNK and p38 MAPK pathways are preferentially activated by inflammatory cytokines and various forms of stress (Denhardt, 1996). The p38 MAPK signaling pathway is involved in lipopolysaccharide (LPS)-induced cyclooxygenase-2 expression in RAW 264.7 macrophages (Paul et al., 1999), J774 macrophages (Chen et al., 1999), and human monocytes (Dean et al., 1999).

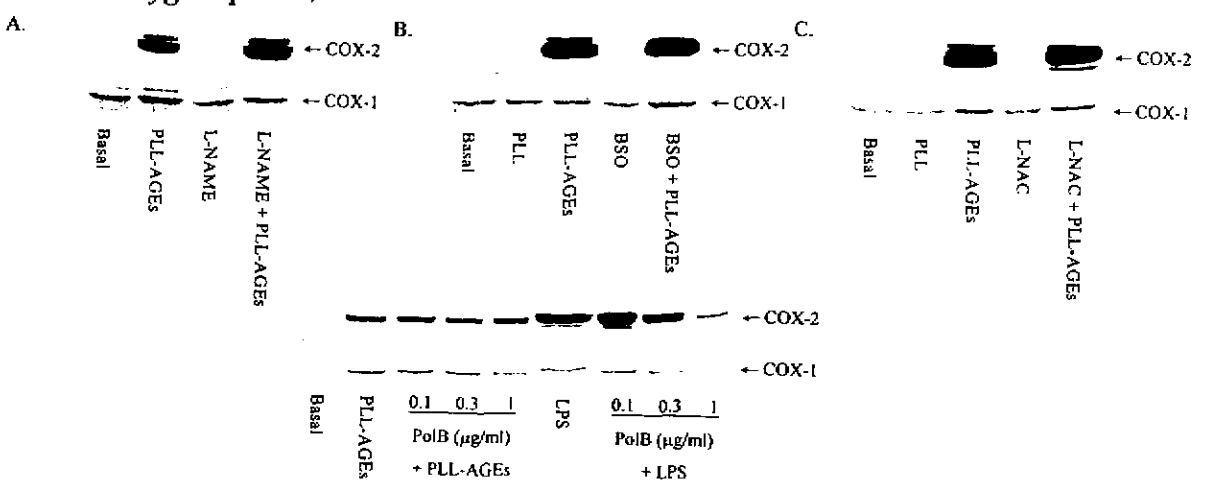
In the present study, we investigated the effect of PLL-derived AGEs on cyclooxygenase-2 expression in the murine macrophage cell line, RAW 264.7. We found that the PLL-AGEs stimulated a dose- and time-dependent up-regulation of both cyclooxygenase-2 protein expression and cyclooxygenase activity. These effects can be blocked by pretreatment of RAW 264.7 cells with either the tyrosine kinase inhibitor, genistein, or the p38 MAP kinase inhibitor, SB 203580, suggesting that the tyrosine kinase-p38 MAP kinase pathway is involved in this signaling event.

三、結果與討論

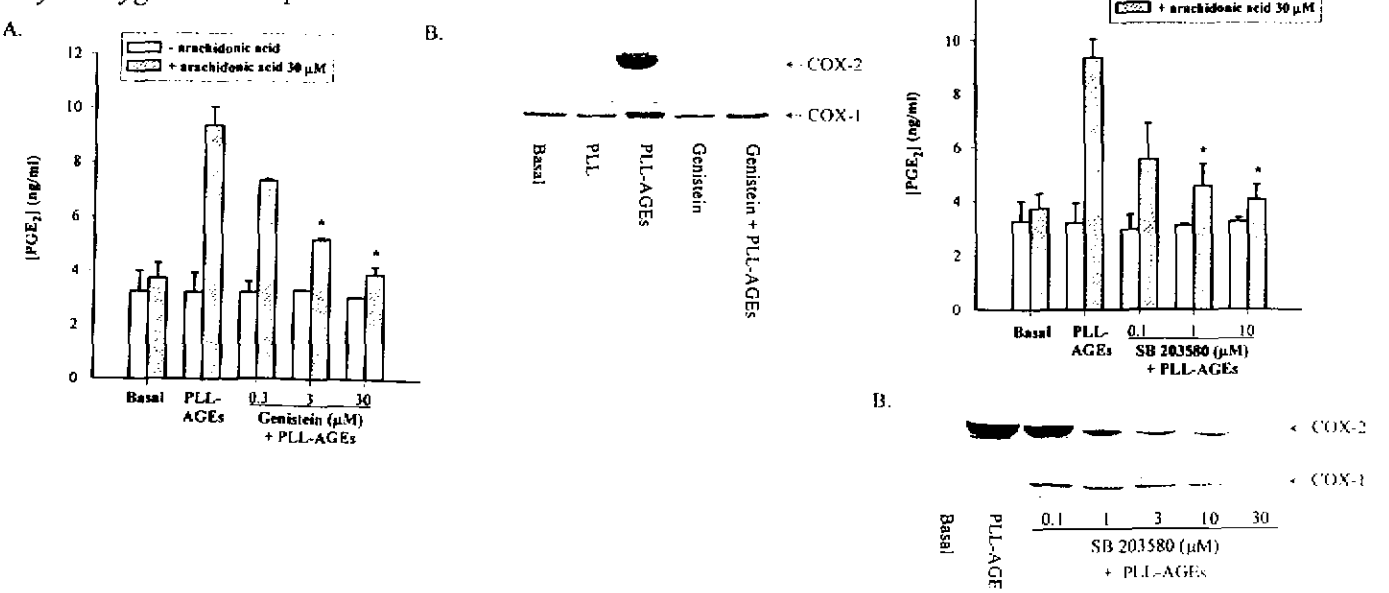
3.1. PLL-AGEs stimulate the increase in cyclooxygenase activity and cyclooxygenase-2 expression



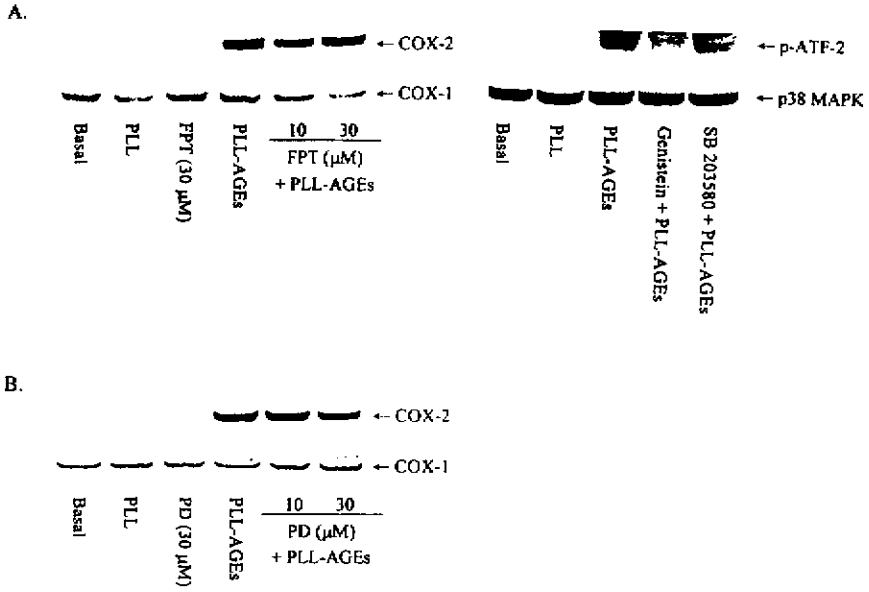
3.2. PLL-AGEs-stimulated cyclooxygenase-2 expression is not due to iNOS induction, reactive oxygen species, or LPS contamination



3.3. Mechanisms by which PLL-AGEs induce the increase in cyclooxygenase activity and cyclooxygenase-2 expression



3.4. PLL-AGEs activate p38 mitogen-activated protein kinase



In conclusion, this study provides evidence for a novel role of AGEs in the regulation of cyclooxygenase-2 expression. Our results raise the possibility that diabetic complications are mediated by AGEs-induced cyclooxygenase-2 expression. It will be of interest to determine whether cyclooxygenase-2 inhibitors can be used to control diabetic squealae.

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 及 European Journal of Pharmacology (In press)。另外有關 PI-PLC, PC-PLC、PKC 亞型的研究，已投稿 Biochemical Pharmacology；PI-3 Kinase、NFkB 的研究結果則也已投稿 British Journal of Pharmacology。除此之外，我們也將過去研發出來的血中 AGEs 自動分析法投稿到 Journal of Clinical Biochemistry。所以今年我們以投稿五篇文章再 SCI 期刊，其中二篇已接受，另三篇正在進行，近期會有消息。

五、參考文獻

Lin CH, Lin YF, Chang MC, Wu CH, Ho YS, and Lee HM, 2001 Advanced glycosylation end products-induced nitric oxide synthase expression in C6 glioma cells: involvement of p38 MAP kinase dependent mechanisms. *Life Sci.69*,2503-2515.

Lin CH, Wu CH, Thum WY, Ho YS, and Lee HM, 2001 Involvement of p38MAPK in PLL-AGE-induced Cox-2 expression and PGE2 production. *Europ. J. Pharmacol.(In press)*.