RRPC90091208 (6 .P)

行政院國家科學委員會補助專題研究計畫成果報告

糖尿病併發腎臟病的致病機轉

計畫類別：□個別型計畫　□整合型計畫

計畫編號：NSC90-2320-B-038-030

執行期間：90 年 8 月 1 至 91 年 7 月 31 日

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

中　華　民　國　91　年　10　月　31　日

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，稱為"過度糖化最終產物" (Advanced Glycosylation End Products)，簡稱為AGEs。因此組織蛋白過度糖化是糖尿病併發症，包括腎臟併發症的主要病因。本研究以AGEs刺激腎臟環間膜細胞，並研究誘導型一氧化氮合成酶(inducible-Nitric Oxide Synthase，簡稱iNOS) 的表現及一氧化氮的累積情形。以BSA-AGEs處理mesangial細胞後，iNOS的表現及NO的釋放明顯增加，我們並證實其訊息傳遞機制和酪氨酸的磷酸化和p38MAPK的活化有關。另外因為PPAR-γ的活化可改善糖尿病性腎病，若以rosiglutazone(PPAR-γ的活化劑)前處理環間膜細胞，則AGEs引起的iNOS表現及NO的釋放均可被抑制，我們並發現rosiglutazone也可抑制因AGEs引起的p38MAPK活化，綜合以上，顯示rosiglutazone影響AGEs引起的iNOS表現及NO釋放是經由抑制p38MAPK的活化所導致。

關鍵詞：過度糖化最終產物、誘導型一氧化氮合成酶、腎臟環間膜細胞、p38 MAPK

Abstract

Advanced glycosylation end products (AGEs) accumulation in tissue have been implicated in diabetic related complications including diabetic nephropathy. Activation of peroxisome proliferator activated receptor-γ (PPARγ) ameliorates diabetic nephropathy. In the present study, we investigated the effects of AGEs on inducible nitric oxide synthase (iNOS) expression and NO production, and the effects of rosiglutazone, an activator of PPAR-γ, on AGE-induced iNOS expression and nitrite release in glomerular mesangial cells. AGEs caused a dose- and time-dependent increase of iNOS induction and nitrite accumulation in mesangial cells. A protein tyrosine kinase inhibitor (genistein), or a p38 mitogen activated protein kinase (MAPK) inhibitor (SB203580) suppressed AGE-induced iNOS expression and nitrite release from mesangial cells. Addition of BSA-AGEs to mesangial cells increased p38 MAPK activities suggesting AGEs may mediate iNOS induction through p38 MAP kinase activation. Activation of PPAR-γ by rosiglitazone inhibited AGE-induced iNOS expression and nitrite release from mesangial cells. Rosiglitazone also blocked AGE-stimulated p38 MAP kinase activation in mesangial cells. These data suggest that rosiglitazone may prevent AGE-induced iNOS expression and subsequent NO production by interfering with p38 MAP kinase activity.

Key Words: AGEs, iNOS, mesangial cells, P38 MAP kinase.

二、緣由與目的

Advanced glycosylation end products (AGEs) are formed by non-enzymatic "Maillard reaction" (1), and have been related to the pathogeneses of diabetic complications and aging (2). In diabetic kidney, AGEs were detected in the mesangial area in glomeruli, which were associated with a paralleled increased of inducible nitric oxide synthase (iNOS)-positive cells and intraglomeruli NO_2^-/NO_3^- production (3). AGEs have been shown to stimulate inducible

nitric oxide synthase (iNOS) expression and nitric oxide release from a variety of cell lines. However, the mechanisms by which AGEs induce iNOS expression in glomerular messangial cells have not been elucidated.

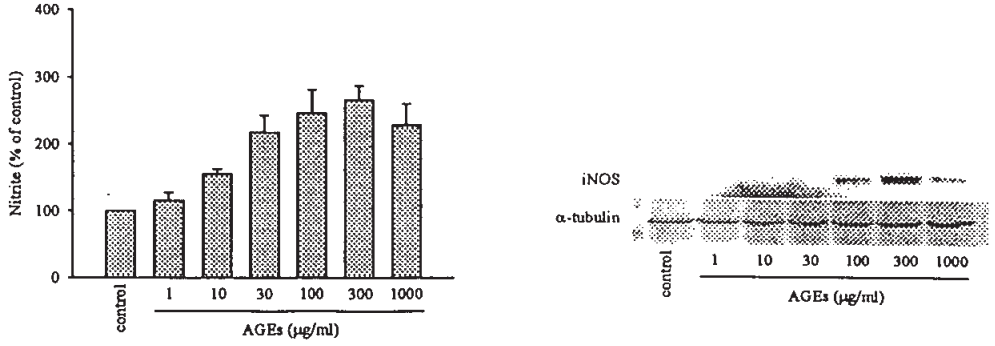
The mitogen-activated protein (MAP) kinases mediate the response of renal glomerular mesangial cells to a variety of physiologic and pathologic stimuli. The expression of iNOS seems to be closely related with the activation of mitogen-activated protein kinases (MAPKs). There are three important groups 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 (4). Engagement of AGE receptors by AGEs activates a p21 Ras and MAP kinase dependent signal transduction pathway (5). We previously demonstrated that AGEs may activate protein tyrosine kinase to induce p38 MAPK activation, which in turn induces iNOS expression in C6 gliom cells and COX-2 expression in RAW 264.7 cells (6). These findings suggest a role for the p38 MAPK pathway as important signaling mechanisms underlysing the AGE-induced iNOS expression in renal mesangial cells.

PPARγ are nuclear hormone transcription factors that regulate gene associated with lipid and glucose metabolism. PPARγ can be activated by the antidiabetic thiazolidinediones (TZDs) and the natural occurring ligand, the J series of prostaglandins. Activation of PPAR-γ ameliorate diabetic nephropathy and reduced mesangial cell DNA synthesis without affecting mesangial cell viability (7). Activation of PPARγ also inhibits the proinflammatory pathways, including the cytokine secretion (8,9) and iNOS expression (10,11) in a variety of cell lines. The murine iNOS promoter contains 24 transcriptional factor binding sites, including those for NF-kB, AP-1, CREB, and the ets family of transcription factors. Some of these transcription factors are regulated by p38 MAPK. However, whether p38 MAPK plays a role in inhibition of AGE-induced iNOS expression by rosilgitazone has not been investigated.

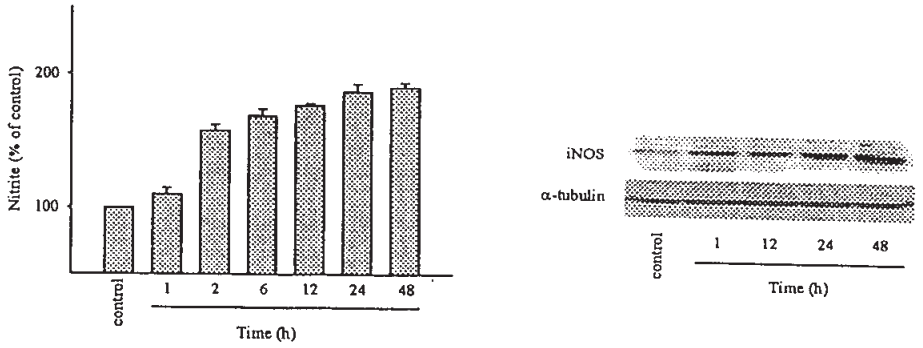
In the present study, we investigate the effects of BSA-AGEs on iNOS expression and NO release in glomerular mesangial cell. We found that BSA-AGEs stimulated a dose- and time-dependent up-regulation of bothe iNOS protein expression and nitrite accumulation. These effects were blocked by pretreatment of messangial cells with either rosiglitazone, the PPARγ activator, or SB203580, the p38 MAPK inhibitor. Given rosiglitazone blocked NO production, iNOS protein expression, and p38 MAP kinase activation in AGE-stimulated mesangial cells, these data suggest that rosiglitazone may prevent AGE-induced iNOS expression and subsequent NO production in mesangial cells by interfering with p38 MAP kinase activation.

三、結果與討論

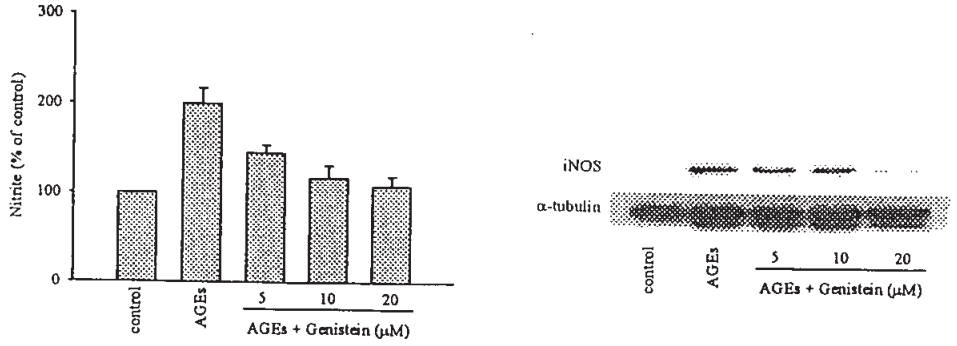
3.1. Concentration -dependent increase of nitrite accumulation and iNOS expression caused by BSA-AGEs in rat mesangial cells.



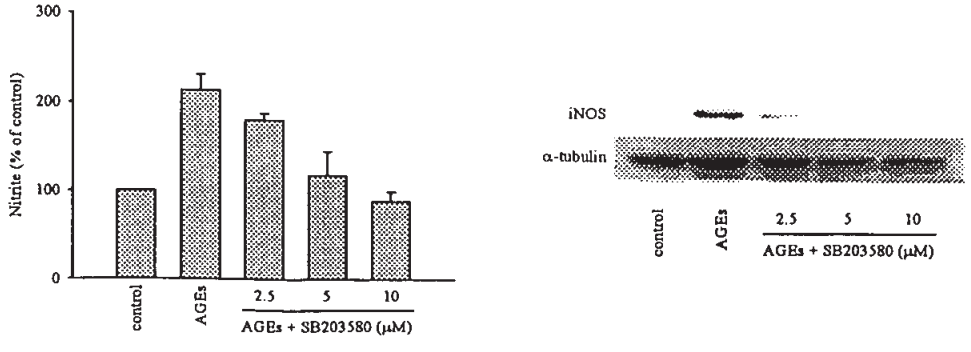
3.2. Time-dependent increase of nitrite accumulation and iNOS expression caused by BSA-AGEs in rat mesangial cells.



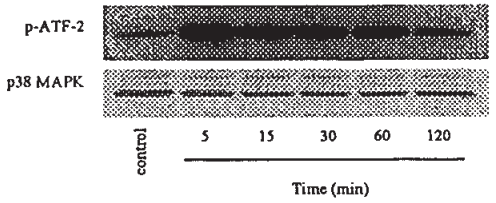
3.3. Effects of the tyrosine kinase inhibitor, genistein, on AGEs-induced iNOS expression and nitrite release from rat mesangial cells.



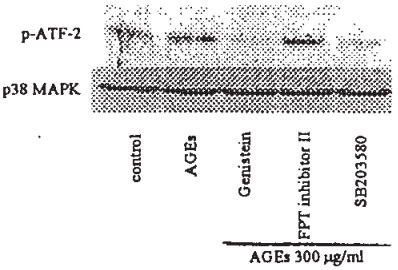
3.4. Effects of the p38 MAPK inhibitor, SB203580, on AGEs-induced iNOS expression and nitrite release from rat mesangial cells.



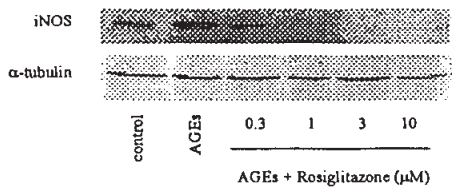
3.5. AGEs activate p38 MAP kinase in rat mesangial cells.



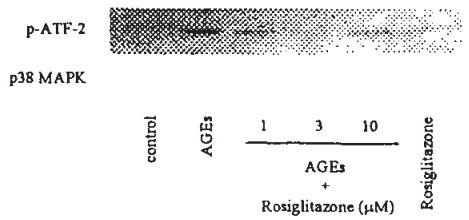
3.6. AGEs stimulated p38 MAP kinase activity was inhibited by genistein and SB203580 but not FPT inhibitor II in rat mesangial cells.



3.7. Effects of the PPAR-γ activator, rosiglitazone, on AGEs-induced iNOS expression from rat mesangial cells.



3.8. Effects of the PPAR-γ activator, rosiglitazone, on AGEs stimulated p38 MAP kinase activity in rat mesangial cells.



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，分別發表於 Life Science 及 Europ J. Pharmacol。另外有關 PI-PLC, PC-PLC、PKC 亞型的研究，已投稿 Biochemical Pharmacology 正在修改；PI-3 Kinase、NFkB 的研究結果則也已投稿 Mol.cell. Endocricol.。除此之外，我們也將研發出來的血中 AGEs 自動分析法發表在 J. Clinical Biochem.。所以今年我們已投稿五篇 SCI 文章，其中四篇已發表，另一篇正在修改（不包括非主要作者的四篇 SCI 文章）。除此之外，我們還有二篇文章正在 review，預計將可順利發表於 SCI 期刊。

五、參考文獻

1. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1988;318:1315-1321.
2. Brownlee M: Glycosylation products as toxic mediators of diabetic complications. Annu Rev Med 1991;42:159-166.
3. Sugimoto H, Shikata K, Wada J, Horiuchi S, Makino H: Advanced glycation end products-cytokine-nitric oxide sequence pathway in the development of diabetic nephropathy: aminoguanidine ameliorates the overexpression of tumour necrosis factor-alpha and inducible nitric oxide synthase in diabetic rat glomeruli. Diabetologia 1999;42:878-886.
4. Denhardt SA: Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signaling. Biochem J 1996;318:729-747.
5. Lander HM, Rauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM: Activation of the receptor for advanced glycation end products triggers a p21 (ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. Biol Chem 1997;272:17810-17814.
6. Lin CH, Wu CH, Thum WY, Ho YS, Lee HM: Involvement of p38 mitogen-activated protein kinase in PLL-AGE-induced cyclooxgenase-2 expression. Eur J Pharmacol 2002;438:143-152.
7. Guan Y, Zhang Y, Schneider A, Davis L, Breyer RM, Breyer MD: Peroxisome proliferator-activated receptor-gamma activity is associated with renal microvasculature. Am J Physiol: Renal Fluid Electrolyte Physiol 2001;281:F1036-1046.
8. Wong A, Dukic-Stefanovic S, Gasic-Milenkovic J, Schinzel R, Wiesinger H, Riederer P, Munch G: Anti-inflammatory antioxidants attenuate the expression of inducible nitric oxide synthase mediated by advanced glycation endproducts in murine microglia. Eur J Neurosci 2001;14:1961-1967.
9. Uchimura K, Nakamuta M, Enjoji M, Irie T, Sugimoto R, Muta T, Iwamoto H, Nawata H: Activation of retinoic X receptor and peroxisome proliferator-activated receptor-gamma inhibits nitric oxide and tumor necrosis factor-alpha production in rat Kupffer cells. Hepatology 2001;33:91-99.
10. Petrova TV, Akama KT, Van Eldik LJ: Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-delta12,14-prostaglandin J2. Proc Natl Acad Sci USA 1999;96:4668-4673.
11. Reilly CM, Oates JC, Sudian J, Crosby MB, Halushka PV, Gilkeson GS: Prostaglandin J(2) inhibition of mesangial cell iNOS expression. Clin Immunol 2001;98:337-345.