中文題目：Pentoxifylline 抑制實驗型急性腎小球間質增生型腎炎機制之探討：腫瘤壞死因子的角色

英文題目：Mechanisms by which pentoxifylline suppresses acute experimental mesangial proliferative glomerulonephritis. Role of tumor necrosis factor-α

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中文摘要

背景：由靜脈單次注射 anti-Thy1 抗體至大鼠體內會迅速誘發急性腎小球間質增生型腎炎產生。巨噬細胞是腫瘤壞死因子 (TNF-α) 的主要來源，分泌腫瘤壞死因子的巨噬細胞在若干實驗型腎炎（如抗腎小球基底膜腎炎、腎毒性腎炎、和 puromycin amino-nucleoside 腎病變）均被証明和腎小球傷害有密切關係。然而在 anti-Thy1 腎炎模式尚未有類似的研究報告。我們最近發現 pentoxifylline 可以降低 anti-thy1 腎炎誘發後第一天至第三天聚集在腎小球內的巨噬細胞數目 (Kidney Int 1999;56:932-943)，吾人假設 pentoxifylline 可能是透過抑制腫瘤壞死因子製造，或是經由抑制巨噬細胞數目而減少腫瘤壞死因子產量，進而減輕 anti-Thy1 腎炎中的腎小球傷害程度。

方法：腎小球 total RNA 是以 guanidine thiocyanate-acid phenol-chloroform 方法抽取，再利用 M-MuLV 反轉錄酵素製成 RT，施行 PCR。北方墨點則按標準分子生物學方法操作。免疫組織化學染色及原位雜合則用來計算浸潤腎小球的單核球及淋巴球數目，以及證明 TNF-α mRNA 存在。

結果：腎炎誘發後兩小時即發現 TNF-α, IL-1β, MCP-1, ICAM-1, VCAM-1 等基因表現增加並達到最高點，RANTES 在腎炎誘發後兩小時也可以 PCR 偵測到 mRNA 增加，但其最高點出現在第二天。原位雜合可偵測到腎小球內 TNF-α mRNA 訊息存在。北方墨點分析發現 pentoxifylline 可降低腎炎誘發後兩小時之腎小球 TNF-α, IL-1β, MCP-1, ICAM-1, VCAM-1 等 mRNA 表現，並伴隨著浸潤腎小球之 ED-1 陽性單核球, CD-4 和 CD-8 陽性淋巴球數目的減少。

結論：這些結果顯示在實驗性 anti-Thy1 腎小球腎炎誘發過程中，腎小球內 TNF-α 與許多沾黏分子和化學趨化因子之基因表現均呈上升。Pentoxifylline 可經由抑制 TNF-α 與這些沾黏分子和化學趨化因子之基因表現達到其抗發炎的效果。

英文摘要

Background Cytokines, chemokines and adhesion molecules play important pathogenetic roles in glomerular leukocyte infiltration in anti-Thy1 nephritis. We previously reported that pentoxifylline (PTX) reduced glomerular macrophage accumulation, ameliorated proteinuria, and attenuated glomerulosclerosis in anti-Thy1 nephritis (Kidney Int 1999;56:932-943). The present study investigates the effect of PTX on the expression of cytokines (tumor necrosis factor (TNF)-α and interleukin (IL)-1β), the C-C chemokines (monocyte chemoattractant protein (MCP)-1 and regulated upon activation, normal T cells expressed and secreted (RANTES)), and adhesion molecules (intercellular adhesion molecule-1, ICAM-1; and vascular adhesion molecule-1, VCAM-1) at 2 h, 1, 2, and 5 days after induction of anti-Thy1 nephritis.

Methods Isolation of glomeruli was performed by graded sievings. Glomerular total RNA

was extracted and first strand cDNA was synthesis by reverse transcriptase. Northern blot analysis and polymerase chain reaction (PCR) was performed according to standard molecular biological procedures. Kidney sections were also processed for immunohistological staining for monocytes/macrophages, CD4- and CD-8-positive T cells using DAB as chromogen substrate. *In situ* hybridization was performed for detection of TNF-alpha mRNA signal within the glomeruli.

Results By reverse-transcript (RT) PCR, the glomerular mRNA level of TNF-α and IL-1β increased appreciably beginning 2 h after administration of the anti-Thy1 antibody. There was also a rapid induction of MCP-1, RANTES, ICAM-1, and VCAM-1 mRNAs in the glomeruli of nephritic rats 2 h after induction of nephritis. *In situ* hybridization detected presence of TNF-alpha mRNA signal within the glomeruli. The administration of PTX decreased glomerular macrophage infiltration while reducing the levels of TNF-α, IL-1β, MCP-1, RANTES, ICAM-1, and VCAM-1 at 2 h. There was also a temporal association between glomerular influx of ED-1 (monocytes/macrophages), CD-4, and CD-8-positive cells and the expression of various cytokines, CC chemokines, and adhesion molecules.

Conclusions These data suggest that PTX may exert its anti-inflammatory effect in anti-Thy1 nephritis by modulating gene expression of TNF-α, as well as multiples chemokines and adhesion molecules within the glomeruli.

計劃緣由

由靜脈單次注射 anti-Thy1 抗體至大鼠體內會迅速誘發急性腎小球間質增生型腎炎產生。這種腎炎的急性期（腎炎誘導後 72 小時內）在病態生物學上的特點包括腎小球間質結構解體和巨噬細胞聚集腎小球間質內。接著大約從誘導後 72 小時起，逐漸出現腎小球間質支持細胞 (mesangial cell) 增生和腎小球細胞外基質沈積現象。這些變化大約在十週左右會自行恢復。由於此模式可於短時間內誘發腎炎，再加上其病態生物學上的細胞變化被研究得相當清楚，因此十分適合做爲研究急性腎炎在病態生理學和治療方面之動物模式。在不同病理類型的人類腎小球腎炎中，巨噬細胞都被發現是腫瘤壞死因子 (TNF-α) 的主要來源。這些分泌 TNF-α 的巨噬細胞在若干實驗型腎炎（如抗腎小球基底膜腎炎、腎毒性腎炎、和 puromycin aminonucleoside 腎病變）均被証明和腎小球傷害有密切關係。然而在 anti-Thy1 腎炎模式尚未有類似的研究報告。

近來有研究顯示 phosphodiesterase 拮抗劑，包括 pentoxifylline ，具有抑制體外細胞增生和細胞外基質產生的作用。我們最近也發現 pentoxifylline 可以降低 anti-Thy1 腎炎誘發後第一天和第三天聚集在腎小球內的巨噬細胞數目，並抑制腎炎誘發後第五天腎小球內間質支持細胞的增生和細胞外基質的基因表達 [1]。這些結果顯示 pentoxifylline 可能具有治療腎炎的潛力。然而其機轉爲何，迄今尚未有過研究。 Pentoxifylline 曾在許多體外細胞實驗中 (包括巨噬細胞) 被証明可以抑制 TNF-α 的製造，腫瘤壞死因子一方面能夠促進腎小球間質支持細胞活化增生，進而分泌沈積細胞外基質 [2, 3]，另一方面又可刺激腎小球間質支持細胞製造單核球化學吸引因子 (monocyte chemoattractant protein-1) 和細胞間沾附分子 (intercellular adhesion molecule-1) ，藉以吸引更多發炎細胞至腎小球內 [4, 5]，因此 pentoxifylline 很可能是透過抑制 TNF-α 製造，或是經由抑制巨噬細胞數目而減少 TNF-α 產量，進而減輕 anti-Thy1 腎炎中的腎小球傷害程度。

目的

本研究的目標包括 (1) 觀察 anti-Thy1 腎炎中腎小球內巨噬細胞是否製造 TNF-α，(2) 探討 TNF-α 在 anti-Thy1 腎炎中所扮演的病態生理角色，(3) 研究 pentoxifylline 減輕

anti-Thy1 腎炎中的腎小球傷害是經由抑制 TNF-α 的製造，還是因爲抑制巨噬細胞數目而導致 TNF-α 總量減少。

結果

As revealed by the RT-PCR assay and Northern blot analysis, gene expression of TNF-α is upregulated, along with interleukin-1β and various CC chemokines (MCP-1, RANTES) and adhesion molecules (ICAM-1, VCAM-1), in the glomeruli early in the course (at 2h) of anti-Thy1 glomerulonephritis. *In situ* hybridization findings showed that the major cellular source for glomerular TNF-α mRNA is likely the infiltrating monocytes/macrophages, rather than neutrophils.

Nephritic rats treated with the cAMP-elevating agent, pentoxifylline, showed decreased mRNA levels of TNF-α, MCP-1, ICAM-1, and VCAM-1, but not of RANTES, within the glomeruli. There was a temporal association between reduction of TNF-α, MCP-1, ICAM-1 and VCAM-1 mRNA levels and decrease in ED-1-positive, CD-4- and CD-8-positive cells within the glomeruli.

討論

The present study demonstrates that a number of proinflammatory cytokines (TNF-α and IL-1β), chemokines (MCP-1 and RANTES), and adhesion molecules (ICAM-1 and VCAM-1) are upregulated during the early phase of the nephritis. These gene products are known to play pivotal roles in mediating influx and accumulation of circulating monocytes within the mesangium and their transformation into tissue macrophages [4-6]. Accordingly, our findings that pentoxifylline suppresses the mRNA levels of TNF-a, MCP-1, ICAM-1, and VCAM-1 provide molecular basis for pentoxifylline's ability to attenuate the severity of glomerular inflammation during the early phase of anti-Thy1 nephritis.

Our *in situ* hybridization findings revealed that mRNA of TNF-α was detected as early as 2 h following induction of the nephritis. Parallel with this finding, RT-PCR also showed that TNF-α message peaked at 2h, remained elevated at 24 h, and then returned to normal by day 5 after induction of the nephritis. It appears that the major cellular source for the production of TNF-α is infiltrating macrophages rather than neutrophils. Whether or not resident glomerular cells also produce TNF-α mRNA remains to be elucidated.

Mechanisms underlying the anti-inflammatory effect of pentoxifylline could be related to its ability to increase intracellular cAMP level. The resultant increase in PKA activity may then suppress NF-κB activity [7]. In anti-Thy1 nephritis, glomerular NF-κB DNA-binding activity has been found to be increased [8]. NF-κB is an important transcriptional activator for a number of genes involved in inflammatory or immune responses. The promotor regions of TNF-α, MCP-1, ICAM-1, and VCAM-1 genes have all been found to contain DNA-binding sequences for NF-κB [9-12]. Pentoxifylline or elevated intracellular cAMP levels has been reported to inhibit NF-κB-mediated transcription in several non-renal cell lines [7, 13]. Wheter or not pentoxifylline may also act by this mechanism to suppress the gene expression of TNF-α, MCP-1, ICAM-1, and VCAM-1 in anti-Thy1 model awaits further study.

計劃成果自評

本計劃在執行中遇到最大的問題在於 neutralizing anti-TNF-α antibody (購自 Genzyme) 無法達到預期改善腎炎的效果。 原因可能包括 (商用) 抗體效價不佳、抗體種類不恰當（如應採用 soluble TNF-α receptor）、給藥時間不適當、或是給藥劑量不足。我們認爲 TNF-α 仍是 anti-Thy1 腎炎中最主要的發炎細胞激素，理由是我們發現在腎炎早期 TNF-α mRNA 即呈明顯上升，而 pentoxifylline 可抑制其基因表現。我們認爲這可能是 pentoxifylline 改善 anti-Thy1 腎炎的主要機轉。未來我們會改採其它方法 (如使用 soluble TNF-α receptor) 來證明這項假說。這些結果將於整理後投稿至相關期刊。

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