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• 計畫中文名稱	脊髓損傷基因機轉研究之動物模式核心設施(I)
• 計畫英文名稱	Project 5--- Gene-Based Spinal Cord Injury Animal Core Unit (I)
• 主管機關	行政院國家科學委員會
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• 執行機構	臺北醫學大學醫學系
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• 使用語言	中文
• 研究人員	楊良友; 許重義 --
• 中文關鍵字	脊髓損傷 動物模式 甲基去氫氧化可體松 糖皮質激素 基因微陣列
• 英文關鍵字	Spinal cord injury (SCI); Animal model; MP ; Glucocorticoid; cDNA microarray
• 中文摘要	脊髓損傷是當前最令醫學界頭痛的問題之一，因爲嚴重的脊髓損傷常常導致患者下肢癱瘓或四肢癱瘓，大幅地降低患者的生產力及生活品質。甲基去氫氧化可體松(methylprednisolone)仍是目前用來治療急性脊髓損傷的唯一合法藥物，它像其他的糖皮質激素一樣，對轉錄因子具有廣泛的作用。對於甲基去氫氧化可體松如何保護脊髓損傷的遺傳機制到目前爲止仍不清楚。本整合型計畫的目標是要揭開甲基去氫氧化可體松保護脊髓損傷機制的神秘面紗。本動物核心設施最主要的使命爲在台北醫學大學建立脊髓損傷的動物模式並有效率地提供脊髓損傷的動物給整合型計畫中的其他子計畫使用。我們已經在相當短的時間內，以 NYU impactor 成功地製造上百隻的脊髓損傷動物提供給其他子計畫使用。除此之外，我們也以基因微陣列的技術來尋找甲基去氫氧化可體松所誘發可能保護受傷脊髓的基因。我們發現在脊髓損傷的動物給予甲基去氫氧化可體松(30 mg/kg)四小時後，在受傷的脊髓某些基因的展現會更加強，這些基因包括 neuropilin (VEGF 的受體)和 lamin B receptor。當然，甲基去氫氧化可體松也抑制了某些基因的展現，而這些基因包括-CSF-induced cysteineprotease (calpain 5) 和 dimethyladenosine transferase。過去的文獻指出在脊髓損傷的動物給予 VEGF 會顯著恢復脊髓損傷動物的功能、減少脊髓損傷的程度及降低脊髓細胞死亡的情形。本計畫的結果及過去的發現支持甲基去氫氧化可體松很可能是經由增加 VEGF 受體的展現而達到保護受傷脊髓的論調。另一方面，細胞凋亡的過程中會有染色質分解的情形發生，而 lamin B receptor 卻在促進染色質的集結上扮演重要的角色。因此，甲基去氫氧化可體松也有可能經由促進 lamin B receptor 的展現而達到保護受傷脊髓的效果。再者，抑制 calpain 的活性也可以促進脊髓損傷的動物恢復某種程度的功能，這意味著甲基去氫氧化可體松也相當可能經由此機制來保護受傷的脊髓。此外，我們也證實甲基去氫氧化可體松可以減輕細胞死亡的程度及增加促進細胞

存活 Bcl-xl 基因之展現。綜合上述的證據，甲基去氫氧化可體松很可能是經由多種途境來達到促進受傷脊髓細胞的存活，進而改善脊髓損傷的狀況。

- 英文摘要

Spinal cord injury (SCI) is one of the most devastating medical conditions. Severe SCI often causes paraplegia or tetraplegia in victims who are usually at young and productive age, affecting quality of life and productivity for life. Methylprednisolone (MP) is still the only approven therapeutic agent for treating acute SCI. MP, like other glucocorticoids, has broad effects on transcription factors (TFs) including those affecting cell viability. The genetic mechanisms underlying MP protection in SCI remain poorly understood. The goal of this PPG aims to unveil the molecular and genetic mechanisms of MP neuroprotection in SCI. This Project serves as the animal core unit, providing SCI animals for other projects. We have acquired the expertise in inflicting SCI in both rats and mice using the well established NYU impactor. In addition to generating SCI animals for other projects in this PPG, we also investigated the gene profiling following SCI with or without MP by using the cDNA microarray technique. Adult female Long Evans rats were randomly assigned to the SCI + Vehicle group or SCI + MP group. SCI at T10 was induced with a trauma dose of 10 g x 25 mm. Vehicle or MP (30 mg/kg) was administered 10 min after SCI in SCI + Vehicle or SCI + MP, respectively. Animals were sacrificed 4 h after vehicle or MP treatment. A 5-mm section of the injured spinal cord was removed and processed for total RNA extraction. The integrity of RNA was examined by Agilent Bioanalyzer before cDNA microarray analysis was performed. Commercial Agilent rat cDNA Microarray chips containing over 14,000 rat cDNAs were used for analysis of gene expression. The 3DNA Array 350TM Array Detection kit for microarrays was used for the cDNA labeling based on the provided protocol (Genisphere Inc., Hatfield, PA, USA) and SubmicroTM hybridization protocol provided by Genisphere was used for hybridization of Agilent cDNA Arrays. A set of genes was up-regulated by MP treatment including neuropilin (vascular endothelial growth factor receptor, VEGF receptor) and lamin B receptor that is involved in chromatin assembly. The expression of another set of genes was inhibited by MP treatment including M-CSF-induced cysteine protease (calpain 5) and dimethyladenosine transferase. Prior studies suggest that VEGF improves behavioral recovery after SCI by protection of blood vessel, inhibition of apoptosis, and potentiation of cell survival (spared tissue), suggesting that MP may protect injured spinal cords via increased expression of neuropilin. Moreover, apoptotic process may involve disassembly of chromatin. MP treatment increased the expression of lamin B receptor that is important for chromatin assembly, raising the possibility that MP treatment may protect cells in injured spinal cord against apoptosis via this mechanism. Other studies have also shown that inhibition of calpain activity promotes functional recovery following SCI. Furthermore, MP treatment decreased the number of apoptotic cells revealed by TUNEL staining and enhanced the expression of Bcl-xl, which promotes cell survival. Together, our findings strongly suggest that MP may exert its neuroprotective effect in SCI via multiple genetic mechanisms that converge on the enhancement of cell viability after SCI.