PMC 與 TMPZ 於暫時性局部腦缺血與再灌流動物模式中神經保護作用之機轉探討

The Neuroprotective Mechanism of PMC and TMPZ in a Transient Focal Ischemia/Reperfusion Rat Model

中文摘要

腦中風為造成全球血管性疾病致死率及發病率主因之一。相關的缺血性腦中風之病理機轉受到廣泛的研究，但有關缺血性腦中風之治療藥物卻沒有相當的進展。神經保護製劑為缺血性腦中風治療藥物之一，但不幸的，臨床上並沒有任何一種神經保護製劑可以成功地避免缺血性腦中風的傷害。由於中大腦動脈為人類缺血性腦中風最容易發生之部位，因此，中大腦動脈缺血再灌流動物模式為目前最普遍用來研究缺血性腦中風藥物治療之利器。

PMC (2, 2, 5, 7, 8-pentamethyl-6-hydroxychromane)為維生素 E (α-tocopherol)中最具清除自由基能力之衍生物。根據近來的文獻指出，PMC 具有抑制血小板凝集及抑制細胞激素刺激 NF-κB 活化之作用。TMPZ (2, 3, 5, 6-tetramethylpyrazine)為中藥川芎(Ligusticum wallichii)之萃取物，具有抗血小板凝集及血管舒張作用，且 TMPZ 曾被證實能夠增加急性腦血栓病患之腦部微循環。

本實驗評估 PMC 及 TMPZ 對於中大腦動脈缺血再灌流動物模式所引起之傷害是否具有保護作用；另外，更進一步的探討藥物是否具有抑制缺血性再灌流傷害所引起之發炎或是細胞凋亡反應的能力。PMC 及 TMPZ (10 及 20 mg/kg)以劑量相關而能夠明顯具有意義地減少 60 %及 59.3 %由缺血再灌流傷害所引起之梗塞面積。接著實驗再進一步從分子層面測試 PMC 及 TMPZ 對此傷害之神經保護機轉，經由西方墨點法及免疫螢光染色法分析後，發現預防性投與 PMC 及 TMPZ 能明顯減少 iNOS、nitrotyrosine、HIF-1α 及 caspase-3 表現；經由反轉錄-聚合酵素連鎖反應方法分析發現，PMC 及 TMPZ 能夠抑制 TNF-α mRNA 表現。除此之外，從大鼠腦部均質液之脂質過氧化試驗中，證明 PMC 直接地抑制鐵離子所造成之過氧化作用，而達到抗氧化的目的，但在 TMPZ 方面卻沒有發現此效應。根據這些結果發現，PMC 及 TMPZ 具有神經保護能力藉以避免腦部缺血再灌流傷害。此效果可能的機轉為透過減少 iNOS 及 peroxynitrite 表現而減少氧化壓力傷害，抑制發炎前細胞激素 TNF-α mRNA 表現而降低發炎反應的進行，也抑制 caspase-3 及 HIF-1α 表現以避免神經細胞凋亡。然而，更精確的神經保護機轉仍待未來利用細胞實驗研究其中更進一步的訊息傳遞反應。

英文摘要

Stroke is one of the leading causes of mortality and morbidity world-wide. Although our knowledge concerning the molecular and cellular pathophysiology of brain

injury after focal ischemia has advanced greatly, the development of new treatment drugs for acute ischemic stroke has not progressed as rapidly. One strategy for treating acute stroke patients is the development of neuroprotective drugs. Unfortunately, clinical trials using various preclinically neuroprotective drugs for stroke have proven unsuccessful. Because the middle cerebral artery is the vessel mostly affected by cerebral occlusion in ischemic stroke, so the middle cerebral artelry occlusion (MCAO) of rodents provides an excellent model that is relevant to ischemic stroke in human.

PMC (2, 2, 5, 7, 8-pentamethyl-6-hydroxychromane) is an analogue of α-tocopherol and has potent free radical scavenging activity. It is known to inhibit platelet aggregation and inhibit the activation of cytokine-induced NF-κB (nuclear factor-κB). TMPZ (2, 3, 5, 6-tetramethylpyrazine) is extracted from the root of Ligusticum wallichii, a common herb used in traditional Chinese medicine. TMPZ has antiplatelet and vasodilation activity. It is shown to improve changes in microcirculation of patients with acute cerebral thrombosis. In this study we evaluated the protective effects of PMC and TMPZ in a cerebral ischemia-reperfusion injury model in rats, and the further inhibitory effects on generation of inflammatory response and expression of apoptosis.

We test the effects of PMC and TMPZ in transient focal cerebral ischemia and reperfusion rat modal. PMC and TMPZ (20 mg/kg ip.) markedly attenuated the infarct volume about 60 % and 59.3 % respectively at 24 hours after middle cerebral artery occlusion. Subsequently, we examined the neuropreotective mechanisms of PMC and TMPZ in the molecular and cellular pathophysiology of brain injury after focal ischemia. By the data of western and immunofluorescent analysis, we found that pretreatment of PMC and TMPZ may significantly reduce the expression of iNOS, nitrotyrosine, HIF-1 α . and caspase-3. By reverse transcription-polymerase chain reaction analysis, PMC and TMPZ also suppressed the expression of TNF-α . mRNA. Moreover, we prove the directly antioxidant effect of PMC, but not TMPZ in the test of lipid peroxidation in rats brain homogenates.

According to these findings, PMC and TMPZ have neuroprotective effects against cerebral ischemia and reperfusion injury. Moreover, the beneficial results may due to the reduction of iNOS, peroxynitrite, the suppression of pro-inflammatoy cytokine TNF-α and the inhibition of apoptotic of caspase-3 and HIF-1α. However, the exact mechanisms of their neuroprotective effects at further signal transduction of cellular level need to be clarified in the future.