• 系統編號	RG9513-3324
• 計畫中文名稱	川芎之活性生物鹼 tetramethylpyrazine 併用 aspirin 對預防缺血性腦梗塞之研究
• 計畫英文名稱	Study the Effect of Tetramethylpyrazine Combined with Aspirin on Ischemic Cerebral Infarction
• 主管機關	行政院衛生署
• 計畫編號	CCMP94-RD-031
• 執行機構	台北醫學大學醫學研究所
• 本期期間	9401 ~ 9412
• 報告頁數	54 頁
• 使用語言	中文
• 研究人員	許準榕；林冠宏；；；； Sheu, Joen-Rong；Lin, Kuan-Hung；；；；
• 中文關鍵字	中大腦動脈；阻塞再灌流；aspirin；TMPZ；；；；
• 英文關鍵字	middle cerebral artery；occlusion/reperfusion；aspirin；TMPZ；；；；
• 中文摘要	本實驗目的在探討 TMPZ 20 mg/kg 併用 aspirin 5 mg/kg 在大鼠的中大腦動脈梗塞再灌流實驗中，是否能有意義加強預防腦受損之效果，以及研究其分子機轉，並且期待能夠降低藥物使用劑量以符合臨床之應用，減少長期服用之副作用產生。 本實驗主要利用大鼠中大腦阻塞再灌流之動物模式，並且利用腦組織去分析相關重要誘導腦受損之因子，包括(1)利用西方墨點法 (Western blotting) 測定腦梗塞引起誘發性一氧化氮合成酵素 (iNOS)、HIF-1alpha 和 caspase-3 之表現含量，(2)利用組織免疫染色法 (Immunohistochemical staining) 觀察腦梗塞後 nitrotyrosine 的生成，(3)腦部脂質過氧化體外試驗(Lipid peroxidation)和(4)反轉錄-聚合酵素連鎖反應(Reverse transcription-polymerse chain reaction；RT-PCR)來偵測 mRNA 的表現含量。 TMPZ (20 mg/kg)能夠明顯具有意義地減少 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 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 TMPZ 能明顯減少 iNOS、nitrotyrosine、HIF-1alpha 及 caspase-3 表現；經由反轉錄-聚合酵素連鎖反應方法分析發現，TMPZ 能夠抑制 TNF-alpha mRNA 表現。除此之外，從大鼠腦部均質液之脂質過氧化試驗中，發現 TMPZ 並不能抑制鐵離子所造成之過氧化作用；另一方面，TMPZ 併用 aspirin 沒有意義加強其作用效果，雖然本實驗並沒有發現 TMPZ 合併 aspirin 有提高腦保護的作用，但是我們推測 TMPZ 及 aspirin 在本實驗所使用的劑量下已到其閥值，所以我們預期如將此兩藥的劑量降低便可觀察到此兩藥合併之效果，而且在臨床上便能夠大大降低因長期服用所產生的副作用，在未來，我們可以利用降低劑量來做更進一步探討 TMPZ 合併 aspirin 對於缺血/再灌流實驗中所造成之影響，以及分子機轉之探討。
• 英文摘要	In this study, we investigated whether TMPZ 20 mg/kg combined with aspirin 5 mg/kg could potentiate their protective effect on the brain injury and

determined their molecular mechanisms in the middle cerebral artery occlusion/reperfusion model in rats. Furthermore, we expected to decrease dosage of TMPZ and aspirin in order to decrease size effect and correspond to clinical application. In addition to analysis of infarct size, we also utilized brain tissue to (1) measure the content of iNOS, HIF-1alpha and caspase-3 by western blotting, (2) study nitrotyrosin formation by immunohistochemical staining, (3) test lipid peroxidation in vitro and (4) analyze TNF-alpha mRNA expression by Reverse transcription-polymerse chain reaction (RT-PCR). We tested the effects of TMPZ in transient focal cerebral ischemia and reperfusion rat modal. TMPZ (20 mg/kg ip.) markedly attenuated the infarct volume about 59.3 % at 24 hours after middle cerebral artery occlusion. Subsequently, we examined the neuropreotective mechanisms of 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 TMPZ could significantly reduce the expression of iNOS, nitrotyrosine, HIF-1alpha and caspase-3. By reverse transcription-polymerase chain reaction analysis, TMPZ also suppressed the expression of TNF-alpha mRNA. Moreover, we found that TMPZ couldn't improve lipid peroxidation in rat's brain homogenates in vitro. On the other hand, our study found that TMPZ combined with aspirin didn't potentiate their protective effect on the brain injury, compared with TMPZ or aspirin alone. We postulated that the dosage of TMPZ or aspirin almost reached optimal protective effect on the brain injury in this study. Therefore, it was hard to discriminate whether TMPZ combined with aspirin could potentiate their protective effect on the brain injury. In the future, we will decrease the dosage of TMPZ and aspirin and this approach will largely decrease size effect and correspond to clinical application.