探討 Puerarin 於中風動物模式之神經保護作用

The neuroprotective mechanism of puerarin in a transient focal ischemia/reperfusion rat model

中文摘要

腦中風一直是國內十大疾病死因之第二位，也是國人失能的主要原因，雖然缺血性腦中風之相關病理機轉受到廣泛研究，但有關治療缺血性腦中風藥物的開發上並無實質的進展，而神經保護製劑爲缺血性腦中風的治療藥物之一，然而，臨床上尚未有任何一種神經保護製劑能夠完全避免缺血性腦中風的傷害。由於中大腦動脈爲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是最常被應於缺血性腦中風藥物研究的動物模式。

Puerarin（4’,7-dihydroxy-8-β-glucosylisoflavone）爲中藥葛根 (Pueraria lobata)的主要活性成份。根據近年來的研究文獻指出；puerarin 具有擴張冠狀動脈、降低體溫以及增加腦部血流的功能，且 puerarin 亦曾被證實能夠改善動物因心血管以及腦血管病變的微循環。

本實驗評估 puerarin 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。另外進一步的探討該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。

實驗結果顯示，puerarin (50 mg/kg)能夠有意義地減少因缺血再灌流傷害所引起之梗塞面積。從分子生物層面測試 puerarin 對神經傷害的保護機轉，經由西方墨點法發現，投與 puerarin 能夠減少 iNOS 和 HIF-1α 蛋白質表現及減少 caspase-3 的活化；經由反轉錄-聚合酵素連鎖反應方法分析發現，puerarin 能夠減少 TNF-α 發炎因子以及 c-fos mRNA 的表現，並能改善 Bcl-2 的 down regulation。除此之外，puerarin 在體外實驗中也發現有抑制嗜中性白血球活性的效果。

然而，在鼠腦均質液之脂質過氧化試驗結果中，puerarin 並無抑制鐵離子所造成過氧化作用；同時，在電子順磁共振(ESR)測試結果中發現 puerarin 在體外並無清除自由基的能力。

根據上述這些發現，証實 puerarin 具有保護神經的能力以減少腦部缺血再灌流的傷害。我們推測此效果的機轉應爲透過抑制 iNOS、HIF-1α、TNF-α 等發炎因子的表現而減少其傷害，抑制嗜中性白血球活化而降低發炎反應的進行。另一方面，puerarin 亦能抑制 caspase-3 以及 c-fos 的表現、減少 Bcl-2 down regulation 進而避免神經細胞的凋亡，然而更詳盡的神經保護機轉仍待進一步的研究

英文摘要

Stroke is still the 2nd leading causes of mortality and morbidity worldwide, it’s also a

major factor to make the Taiwanese death and disability.

Although our knowledge concerning the molecular and cellular pathophysiology of brain injury after focal ischemia has advanced greatly, the development of new drugs for acute ischemic stroke has not progressed as rapidly. One strategy for treating acute stroke patients is the development of neuroprotective drugs.

Unfortunately, the clinical trial of using various preclinically neuroprotective drugs for stroke has fail. The middle cerebral artery is the vessel mostly affected by cerebral occlusion in ischemic stroke, thus the middle cerebral artery occlusion (MCAO) of rodents provides an excellent model that is relevant to ischemic stroke in human. Puerarin（4’ ,7-dihydroxy-8-β-glucosylisoflavone）is a major active compound extracted from the root of Pueraria lobat, a traditional Chinese medicine. It has been shown to improve microcirculation of cardial and cerebral diseases. In this study, we evaluated the protective effects of puerarin in cerebral ischemia-reperfusion animal injury model.

We found that puerarin (50 mg/kg, ip) markedly attenuated the infarct volume at 24 hours after MCA occlusion/reperfusion. Puerarin pretreatment can significantly reduce protein expression of iNOS and HIF-1α, and inhibit caspase-3 activation in the experiment of Western blotting. Using reverse transcription-polymerase chain reaction analysis,puerarin suppressed the expression of TNF-α mRNA and c-fos, and also decreased Bcl-2 downregulation in the damage cerebral cortex area. On the other hand, we also found that puerarin could inhibit fMLP-induced neutrophils activation in vitro. However, our studies revealled that puerarin has no activities in antioxidantion and free radical scavenging.

In conclusion, we demonstrated that puerarin had neuroprotective effects against cerebral ischemia/reperfusion injury in rats, may involve in : (1) reduction of iNOS and HIF-1α protein expression, (2) suppression of TNF-α and c-fos mRNA expression, (3) prevention of Bcl-2 down regulation, (4) inhibition of caspase-3 activation in vivo and (5) attenuation of fMLP induced-neutrophil activation in vitro. However, the more detailed neuroprotective mechanisms of puerarin will be further investigated in the future