• 系統編號	RG9903-0002
• 計畫中文名稱	建立體外腦內皮細胞和體內動物腦損傷模式以評估健康食品對腦血管功能的保健功效
• 計畫英文名稱	--
• 主管機關	行政院衛生署
• 計畫編號	DOH098-TD-F-113-098010
• 執行機構	台北醫學大學醫學研究所{台北醫學大學醫學科學研究所}
• 本期期間	9803 ~ 9902
• 報告頁數	54 頁
• 使用語言	中文
• 研究人員	陳瑞明；林怡伶；謝惠姍；；； Chen, Ruei-Ming；Lin, Yi-Ling；Hsieh, Hui-Shan；；；
• 中文關鍵字	健康食品；腦血管；血腦障壁；腦內皮細胞；；；；
• 英文關鍵字	health food；brain tissue；blood-brain barrier；cerebrovascular endothelial cells；；；；
• 中文摘要	本研究計劃旨在建立一體外(in vitro)腦內皮細胞(cerebrovascular endothelial cells)和體內(in vivo)腦損傷模式，以探討健康食品白藜蘆醇(resveratrol)對於血腦障壁(blood-brain barrier)可能具有的保健功效。本計畫已順利自 ICR mice 大腦微血管取得腦內皮細胞，而且經由 vimentin 和 factor VIII 的免疫組織分析證實，我們所取得的皮細胞確爲腦內皮細胞且純度大於 90%以上。由進一步以此腦內皮細胞爲研究模式發現，健康食品白藜蘆醇或 ascorbic acid 能夠保護腦內皮細胞免於 menadione 和 t-BHP 所導致的氧化性傷害(oxidative insults)。而由死亡機轉(death mechanism)的實驗證實，白藜蘆醇或 ascorbic acid 所引起的保護機制是透過抗細胞凋亡的路徑(antiapoptotic pathway)。於此同時我們也發現，白藜蘆醇能夠降低 menadione 所引起細胞凋亡相關蛋白(apoptosis-related proteins)如 Bcl-2、Bax 和 cytochrome c 在腦內皮細胞的表現量。本計畫也進行腦內皮細胞 tight junction 功能的研究，結果發現健康食品白藜蘆醇或 ascorbic acid 能回復 menadione 或 t-BHP 所改變的 tight junction permeability 和 transendothelial electrical resistance 數值。此外，爲了驗證 in vitro 細胞實驗的結果，本計畫也分別建立 menadione 腹腔注射和中腦動脈栓塞(MCAO)的動物模式，探討健康食品白藜蘆醇或 ascorbic acid 對血腦障壁可能具有的保健功效。研究結果顯示，menadione 和 MCAO 會造成血腦障壁的滲漏(leakage)，因而會讓 Evans blue 染劑通過血腦障壁而到達神經組織。然而健康食品白藜蘆醇或 ascorbic acid 則可以保護血腦障壁免於 menadione 或 MCAO 所造成的血腦障壁傷害。此外此一實驗結果，也經由共軛焦顯微鏡分析法得到更進一步的證實。而經由機轉探討也顯示，白藜蘆醇對血腦障壁所顯現的保護機轉，是透過降低 menadione 和 MCAO 對 ZO-1 聚合(polymerization)所引起的傷害所致。同時，白藜蘆醇也能降低腦神經組織因 menadione 和 MCAO 處理所造成的 DNA 斷裂(DNA fragmentation)和細胞凋亡性損傷(apoptotic insults)。所以由本計畫所建立的體外與體內研究模式證實，白藜蘆醇能保護腦內皮細胞免於 menadione 和 t-BHP 所引起的傷害，而作用機轉則是透過活化內生性粒線體相關的抗凋亡路

	徑(intrinsic mitochondrion-dependent antiapoptotic mechanism)。白藜蘆醇也能經由降低腦內皮細胞的 tight junction 傷害，以達到保健血腦障壁的功效。經由此一體外和體內研究模式的建立，當可新增健康食品保護功效的評估項目和方法，以做爲更快速分析健康食品對於腦組織尤其是血腦障壁是否具有保護作用的平台。
• 英文摘要	This study was designed to establish in vitro and in vivo models to evaluate the potential of health food on protection against insults to the blood-brain barrier (BBB). We have successfully isolated cerebrovascular endothelial cells (CECs), and these cells were identified by immunocytochemical analyses of vimentin and factor VIII. We used CECs as the experimental model and found that heath food resveratrol and ascorbic acids could decrease menadione- or t-BHP-induced cell death via antiapoptotic mechanisms. Simultaneously, resveratrol could alleviate menadione-caused alterations in the levels of cytosolic apoptosis-related proteins such as Bcl-2, Bax, and cytochrome c. In addition, resveratrol or ascorbic acid could lower menadione-induced breakage of the CEC tight junction permeability and transendothelial electrical resistance. These results were further confirmed by analyses of in vitro animals. Repeated administration of intraperitoneal injection with menadione or MCAO treatment led to leakage of the BBB. However, resveratrol could decrease menadione- and MCAO-induced interruption of the BBB. Our mechanism study has shown that the resveratrol-involved protection occurred through reducing the damage of ZO-1 polymerization induced by menadione and MCAO. Consequently, resveratrol attenuated menadione-caused DNA fragmentation and apoptotic insults to brain neurons. The MCAO-induced necrotic death of brain tissues was significantly descended following administration of resveratrol. Therefore, this study uses our in vitro CEC and in vivo animal models to show that resveratrol can protect the BBB against oxidative stress- or MCAO-induced insults. And, the mechanisms are due to the suppression of CEC death and function. The establishments of CEC and brain injury models are beneficial to evaluate the effects of heath food on the BBB.