白藜蘆醇保護腦血管內皮細胞免於氧化態低密度脂蛋白傷害之機制研究

PROTECTIVE EFFECTS OF RESVERATROL ON CEREBROVASCULAR ENDOTHELIAL CELLS FROM OXIDIZED LOW DENSITY LIPOPROTEIN-INDUCED INSULTS

中文摘要

腦血管內皮細胞(cerebrovascular endothelial cells；CECs)是血腦屏障(blood brain barrier)的組成之一，血腦屏障能避免有害物質侵入腦部引起傷害。氧化態低密度脂蛋白(oxidized low density lipoprotein；oxLDL)已被證實會傷害血管內皮細胞，進而造成粥狀動脈硬化(atherosclerotic)，但對腦血管內皮細胞的影響則尚未被證實。白藜蘆醇(resveratrol)是一種存在葡萄中的多酚類物質，具抗氧化功能並對預防或改善心血管相關疾病有作用，但是能否保護血腦屏障免於 oxLDL 的傷害則尚無相關研究。本研究將以小鼠 CECs 爲研究模式探討白藜蘆醇如何保護 CECs 免於 oxLDL 的傷害，及其可能機制。

首先以人的 LDL (Low density lipoprotein；LDL)用不同濃度的硫酸銅於不同時間點下培養，偵測氧化程度的差異，結果發現硫酸銅濃度越高，處理時間越長，則會使 LDL 氧化越嚴重。利用 3-(4,5-dimethylthiazol-2-yl)-2,5－diphenyltetra -zolium bromide (MTT)色度分析法來檢驗細胞粒腺體內膜琥珀酸去氫?(succinate dehydrogenase)的活性，發現 200 μg/ml 的 oxLDL 處理 CECs 48 小時會使其存活率降低至 58.7 %，同時觀察到 CECs 型態由原先的紡錘狀皺縮爲圓形、以 trypan blue 染色也發現活性下降 45.9 %，並有 DNA 片段化現象的出現，證實 oxLDL 具有引發 CECs 細胞凋亡(apoptosis)的能力。再以反轉錄聚合?連鎖反應(reverse transcriptase-polymerase chain reaction)方法分析 CECs 內 oxLDL 的受體(lectin-like oxLDL receptor-1；LOX-1) mRNA，發現 CECs 與 oxLDL 共同培養後，LOX-1 mRNA 的表現量上升。此外由 2’,7’- dichlorofluorescin diacetate (DCFHDA)偵測細胞中反應性氧分子(reactive oxygen species；ROS)的含量，利用西方點墨法(western blotting)以及 caspase 酵素活性分析進一步證明細胞凋亡的機制是透過減少 bcl-2 使粒腺體膜通透性上升，增加 bax 與 cytochrome c 蛋白表現與活化 caspase 9、caspase 3、caspase 8。

白藜蘆醇在非細胞實驗中顯示具有抑制 LDL 氧化的效果，10 μM 的白梨蘆醇並不影響 CECs 的型態和存活率。將 CECs 同時處理 200 μg/ml 的 oxLDL 與 10 μM 的白藜蘆醇後發現，細胞型態仍呈現紡錘狀、細胞存活率由 54.1 % 回升爲 83.6 %，另外 DNA 裂解程度減低，證明白藜蘆醇對 oxLDL 造成的細胞凋亡有保護的作用。進一步探討保護作用的機制，發現 LOX-1 mRNA 的表現不

受白藜蘆醇影響，白藜蘆醇對抑制 ROS 的產生、降低粒腺體膜通透性、減少 cytochrome c 蛋白量的表現與抑制 caspase 9、caspase 3 及 caspase 8 的活性均有作用。

由本研究成果可知，白藜蘆醇保護 CECs 的機制並非透過影響 oxLDL 進入細胞的過程，而是在 LDL 的氧化過程中藉由本身抗氧化能力減輕脂質氧化程度，並降低因爲 oxLDL 的進入導致上升的 ROS，減輕其對細胞產生的破壞。不但會調控外生性的細胞凋亡路徑減少 caspase 8 產生；也影響內生性細胞凋亡路徑，減少 bax 的產生，增加 bcl-2 使粒腺體膜通透性上升、cytochrome c 釋出量減少、caspase 9 及 caspase 3 活性降低，來對 CECs 達到保護的作用。

英文摘要

Cerebrovascular endothelial cells (CECs) are the major component cells in the blood brain barrier. Blood brain barrier protects the neurons from toxicants, and thus prevent the further injury. Oxidized low density lipoprotein (oxLDL) has thus been considered to be a major culprit that induces dysfunction of the endothelial cells associated with the initiation of the atherosclerotic lesions. However the effects of CECs elicited by oxLDL have not been elucidated. Resveratrol is a phytoalexin found in grapes and other herbs that has antioxidant and cardioprotective effects, but the protective effects of resveratrol on oxLDL-induced blood brain barrier disruption is not determined. In this study, we attempted to evaluate the protective effects of resveratrol on CECs from oxLDL-induced insults and the possible mechanism. Human LDL was oxidized to different levels (measured by thiobarbituric acid reactive substance) with CuSO4. We showed CuSO4 is in a time- and concentration-dependent manner. Cell mitochondria succinate dehyrogenase viability was assayed by a 3-(4,5-dimethylthiazol -2-yl) 2,5-diphenyltetrazolium bromide (MTT) colorimetric method. The results showed that viable cells decreased to 58.7% after exposure of CECs to 200 μg/ml oxLDL for 48 hours, and apoptosis was demonstrated by using trypan blue exclusion assay, cell morphology change and DNA fragmentation. Moreover analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) showed oxLDL increased lectin-like oxLDL receptor-1 (LOX-1) mRNA production. In addition, it was also found that oxLDL induced apoptosis increasing with reactive oxygen species (ROS) and bax production, loss of mitochondria membrane potential, and cytochrome c released from mitochondria to cytosol, further activated caspase-9、caspase-3 and caspase-8.

In cell free experiment, resveratrol inhibited LDL oxidation. Exposure of CECs to 10 μM resveratrol for 48 hours had no effect on cell viability. Moreover, co-treatment CECs with 200 μg/ml oxLDL and 10 μM resveratrol reduced the apoptosis of CECs. All of the evidences showed that resveratrol protected CECs from oxLDL-induced

injury. Resveratrol inhibited the production of ROS and bax, the loss of mitochondria membrane potential, the release of cytochrome c from mitochondria to cytosol and the activation of caspase-9、caspase-3 and caspase-8. On the other hand, data from RT-PCR analysis showed that resveratrol did not influence LOX-1 mRNA production. Our findings suggested that resveratrol prevented CECs from oxLDL-caused apoptosis. The effects is not via LOX-1 mRNA production but through reducing ROS and bax production, maintaining mitochondria membrane potential, decreasing cytochrome c release, and inhibition of caspase-9、caspase-3 and caspase-8 activation.