Icaritin reduces *tert*-butylhydroperoxide-induced cell damage through activation of Nrf2 and Akt in H9c2 cardiomyoblasts

by

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碩士學位論文

淫羊藿素的心肌保護的作用

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Abstract

Icaritin reduces *tert*-butylhydroperoxide-induced cell damage through activation of Nrf2 and Akt in H9c2 cardiomyoblasts

by Lei Si Wan

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Chinese Medicinal Science

Overproduction of reactive oxygen species (ROS) contributes to oxidative stress which plays a critical role in different heart diseases. The significant intracellular process of oxidation damage includes the distrubed membrane integrity to release lactate dehydragenase (LDH), loss of mitochondrial membrane potential (ΔΨm), and activate array of defensive genes. We investigated the cardioprotective effect against oxidative stress and also the mechanisistic action of icaritin which is an active flavonoid from traditional Chinese herb *Epimedii*.

Firstly, we found that icaritin pretreatment could promote cell survival and attenuate excessive production of ROS in H9c2 exposed to *t*-BHP. Icaritin also protected cell membrane integrity and prevented collapse of mitochondrial membrane potential. To address the involved signaling, we showed that icaritin-treated H9c2 cells induced translocation of Nrf2 and phosphorylation of Akt was induced by icaritin. Western blot results demonstrated that icaritin pretreatment could time-dependently increase nuclear translocation of Nrf2 and activate HO-1 protein expression.Moreover, the cardioprotective effect of icaritin was impaired by

Nrf2 siRNA and AktIV inhibitor, which subsequently suppresses icaritin-induce activation of Nrf2 and HO-1. Addition, icaritin can also prevent Ca^{2+} overload in the progression of *t*-BHP induced apoptosis. In conclusion, icaritin demonstrated an antioxidative effect on *t*-BHP-induced oxidative stress in H9c2 cardiomyocytes mediated by Nrf2 and Akt.

Keywords:

Iacritin, *tert*-butylhydroperoxide, H9c2, oxidative stress, Nrf2, Akt

摘要

過量的氧化應激(reactive oxygen species, ROS)於細胞中產生最終會形成氧化壓力(oxidative stress)，威脅組織功能對器官造成損害，其中在多種心臟及血管的病變扮演着重要角色。氧化壓力能夠對細胞造成明顯的損毀，包括令細胞膜失去完整性釋放出細胞質中的乳酸脫氫酶(lactate dehydrogenase, LDH)，影響線粒體的膜電位(ΔΨm)，以及活化抗氧化損化系統調節一系列的抗氧化劑的表達。

淫羊藿素(icaritin)是傳統中藥淫羊藿(Herb *Epimedii*)其中之一的活性成份，屬黃酮類化合物。本次實驗主要研究淫羊藿素對抗氧化損傷對保護心肌細胞的抗氧化作用及其機制。將 H9c2 大鼠心肌細胞以淫羊藿素預處理後，利用 *t*-BHP 誘導 H9c2 細胞產生氧化應激，發現預處理後能夠增加細胞的存活率，減少 *t*-BHP 誘導 H9c2 細胞內產生過量的氧化活性物，亦能同時保護細胞膜的完整性，和預防線粒體膜電位下降。

對於淫羊藿素的作用機制，在淫羊藿素處理 H9c2 細胞後分別提取總蛋白與核蛋白，其中 Nrf2 轉錄因子發生核轉入，並上調 HO-1 抗氧化酶的表達，及明顯增加 Akt 的磷酸化。利用 Nrf2 siRNA 和 AktIV 抑制劑分別干擾淫羊藿素活化 Nrf2 的核轉入及 Akt 的磷酸化，能夠有效地抑制淫羊藿素對心肌保護的作用。此外淫羊藿素還能夠降低 *t*-BHP 引起的細胞內鈣含量異常增多，避免導致細胞結構損傷和功能代謝障礙。結論 淫羊藿素通過活化 Nrf2/HO-1 信號傳導通路，減輕細胞的氧化應激而達到對心肌細胞的保護作用。

[關鍵字] 淫羊藿素；氧化損傷；心肌細胞；Nrf2；HO-1

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