ƒ ‹ F | Œfia‹ ˙'e›ß• |–M^D‹ ª¤s›p e ƒ¤"G‡ł§i

„«§ fl ˜ · "«""/ƒ ‹ IIA fl fl fl fl fl fl fl fl fl fl 1 "/"" †{

»P»P»P»P»P»P»P»P»P»P»P»P»P»P»P»Pfl fl »Pfl fl –2 "/"" †{

› p e ^§O›¡§O«‹› pe

› pe‰ ,s„¡GNSC94–2314–B–038–032–

ı ƒ ·`¶G¡94 ƒ~08 ⁄ ° 01 ⁄ ØØ 95 ƒ~07 ⁄ ° 31 ⁄ Ø

ƒ ‡ ƒ ¡G» O ¥ _ ´ ˙ ƒ ˙´ ˙¤t

› pe¥ D ø⁄¡HG‡ fl §§

‡ §i^ «‹¡G" ° †‡ §i

‡ B † zŁ¡fG¡¥»› pe¥ i ⁄ ‰ ¶‹ d,

»P ¥` Œ 95 ƒ~9 ⁄ ° 18 ⁄ Ø

一 中英文摘要：

丹參酮 IIA（tanshinone IIA）是由鼠尾草屬植物 (Salvia miltiorrhiza) 的乾燥根莖所純化的雙帖烯(Diterpene)，丹參酮IIA有抗發炎作用和影響粒線體的電子傳遞。本研究說明丹參酮IIA可增加細胞反應性含氧物種(reactive oxygen specis，簡稱ROS)，而ROS的增加確實已被證時可以誘導巨噬細胞（macrophage）內血紅素氧合酶-1(hemooxygenase-1，簡稱HO-1)的表現。我們進一步發現在RAW264.7細胞中，丹參酮IIA能抑制細菌脂多糖(lipopolysacchardide，簡稱LPS) 所誘導的一氧化氮合成脢(inducible nitric oxide synthase，簡稱 i-NOS)和環氧脢-2 (cycloxygenase-2，簡稱COX-2) 的表現。抑制血紅素氧合酶-1(HO-1)或去除一氧化碳(CO)的產生能還原丹參酮IIA所抑制細菌脂多糖體誘導的iNOS表現。因此本研究可說明HO-1參與丹參酮IIA的抗發炎作用。

關鍵詞: 丹參酮 IIA, 環氧脢-2, 血紅素氧合酶-1

Abstract

Tanshinone IIA is a direrpene isolated from Salvia miltiorhiza root. Tanshinone IIA exerts anti-inflammatory effects and influences electron transfer reaction in mitochondria. In the present study, we demonstrated that tanshinone IIA increased intracellular production of reactive oxygen species (ROS), which in turn induces heme oxygenase-1 (HO-1) expression in RAW 264.7 macrophages. Tanshinone IIA inhibited COX-2 and iNOS expression in lipopolysaccharide-activated RAW 264.7 macrophages. Inhibition of HO-1 or scavenging of CO significantly reversed the inhibition of LPS-stimulated nitrite accumulation by tanshinone IIA, suggesting a novel role of HO-1 in the anti-inflammatory effect of tanshinone IIA.

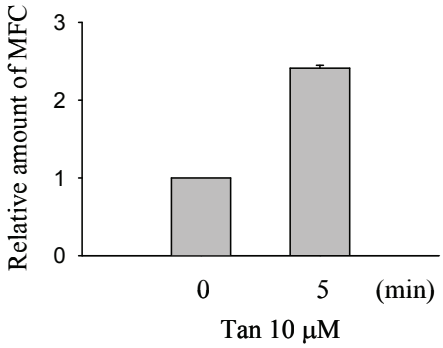
Keywords: Tanshinone IIA, Cyclooxygenase-2; Heme oxygenase-1.

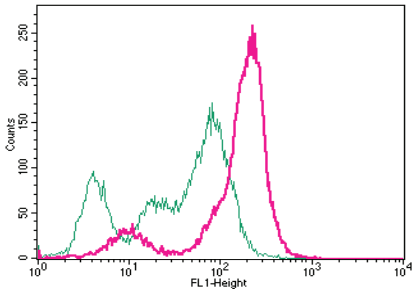
二.緣由與目的

丹參是鼠尾草屬植物(Salvia miltiorrhiza)的乾燥根莖，中醫常用於去瘀生新，可用於治療丹毒、瘡疥；是常用的抗發炎藥物之一。丹參酮IIA（tanshinone IIA）是從丹參純化的雙帖烯(diterpene)，雖然已有數十篇SCI論文探討其藥理功能，但是在細胞及分子階層的研究仍非常不足。本研究的目的即爲探討丹參酮IIA抑制巨噬細胞內誘導型一氧化氮合成脢(簡稱i-NOS)及環氧脢-2 (簡稱COX-2) 的表現。所以我們擬進一步探討誘導HO-1的表現或移除一氧化碳 對丹參酮IIA抑制細菌脂多糖(簡稱LPS)所誘導的iNOS表現有何影響。我們認爲或許丹參酮IIA可增加細胞反應性含氧物種(簡稱ROS)，而ROS的增加確實已被證時可以誘導HO-1的表現。因此本研究除了將可說明單參抗發炎作用的分子機制之外，也將有助於了解HO-1基因表現的調控機制和發炎媒介物質如何被HO-1所調控。

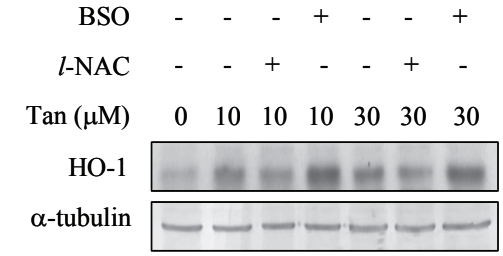
三.結果與討論

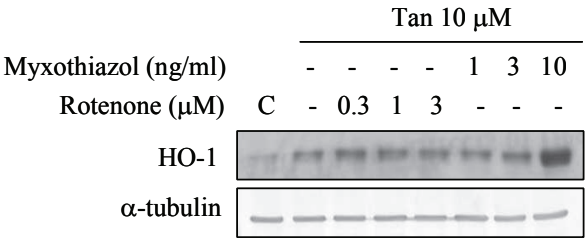
Tanshinone IIA induces HO-1 expression via ROS generation in RAW 264.7 macrophages

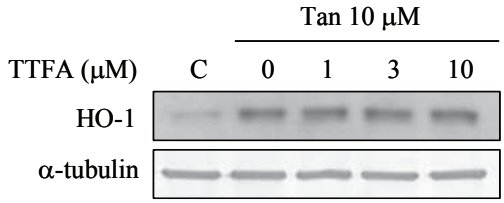


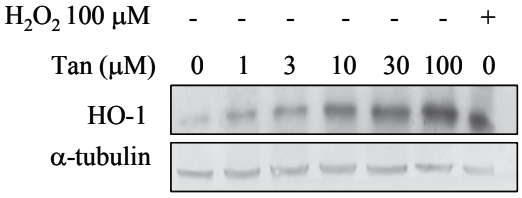


Green : Control
Red : Treat with Tan for 5 min

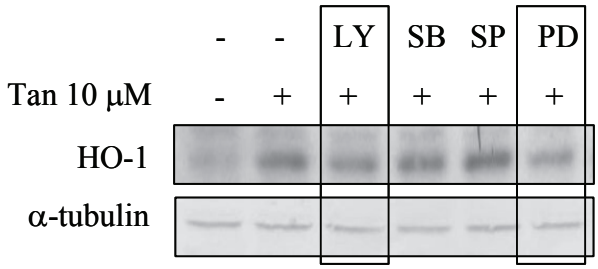


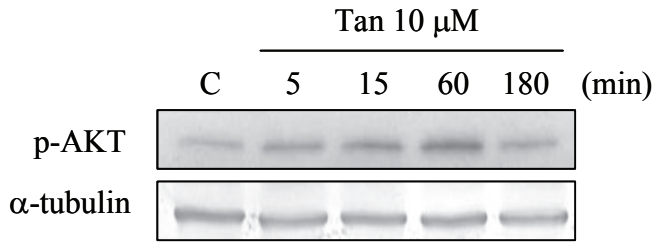


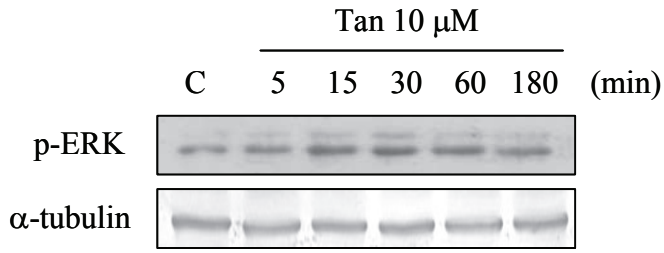




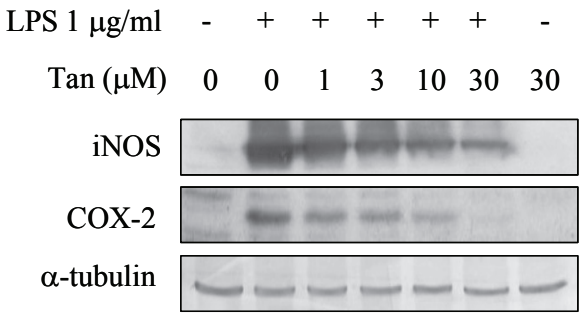
Signaling pathways of tanshinone-induced HO-1 expression in RAW 264.7 macrophages

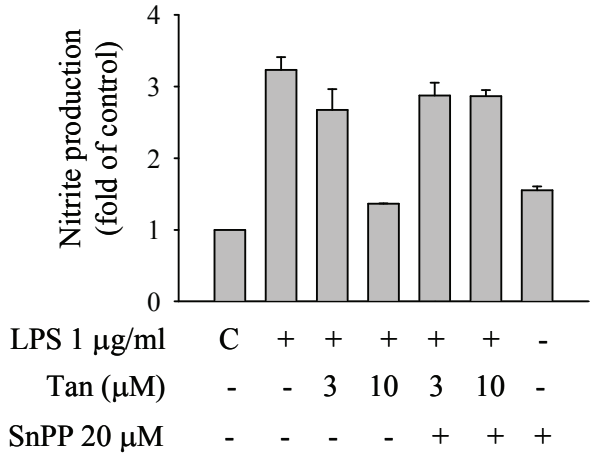


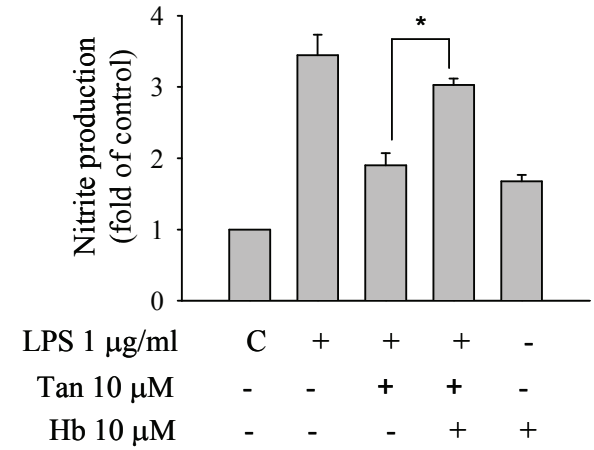




Induction of HO-1 by Tanshinone IIA leads to inhibition of LPS-induced iNOS and COX-2 expression







討論

Danshen' has long been used as an anti-inflammatory agent in China. In the present study, we provided evidence that tanshinones may induce HO-1 expression. The induction of HO-1 expression was mediated through reactive oxygen species, which lead to activation of PI 3-K, and ERK signaling pathways in RAW 264.7 macrophages. In addition, we demonstrated that CO is the key molecule mediating the anti-inflammatory effect of HO-1. We showed that scavenging of CO by hemoglobin attenuated the inhibition of tanshinones on LPS-stimulated nitrite accumulation in RAW 264.7 macrophages.

Tanshinone IIA has been shown to mediate electron transfer reaction in rat heart mitochondria (Zhou et al., 2003). Incubation of RAW 264.7 macrophages with tanshinone dramatically increased intracellular ROS production measured by DCFDA, a ROS-sensitive fluorescent probe. However, inhibition of ETC complexes did not affect the tanshinone-induced HO-1 expression. Instead, increasing glutathione concentrations by the glutathione precursor, *l*-N-acetyl-cysteine, inhibited HO-1 induction; and decreasing glutathione by the gamma-glutamylcysteine synthetase inhibitor, l-N-buthionine-[S,R]-sulfoximine, potentiates the tanshinone-induced HO-1 expression. These data support the notion that tanshinones may increase HO-1 gene expression through ROS production.

HO-1 gene is a prototypical phase II enzyme. Transcriptional activation of HO-1 gene requires binding of transcription factor to the antioxidant responsive elements (AREs) in the promoter proximal region of HO-1 gene. The AREs can be regulated, at least in part, by the Nrf-2 protein. Given activation of PI 3-K may increase the Nrf2

protein level in nuclear, activation of PI 3-K /Akt signaling pathway may mediate HO-1 induction (Nguyen et al., 2003). Consistent with this finding, we found that tanshinone increased Akt phosphorylation in RAW 264.7 macrophages, and inhibition of PI 3-K pathway by specific inhibitor blocked the tanshinone induced HO-1 expression. We also showed that the ERK pathway may be involved. It is not unusual that multiple signaling pathways may converge on HO-1 transcription to mediate their antioxidant activities.

In conclusion, this study provides evidence for a novel role of tanshinone in the regulation of HO-1 expression. Our results raise the possibility that the anti-inflammatory effects of tanshinone are mediated by tanshinone-induced HO-1 expression.

四.計畫成果自評

本研究計畫完成之工作項目有三，(一)丹參酮 IIA抑制細菌脂多糖體所誘導之誘導型一氧化氮合成脢(iNOS)的機制 (二)丹參酮IIA誘導血紅素氧合酶-1 (HO-1)表現的訊息傳遞路徑，(三)丹參酮 IIA透過誘導血紅素氧合酶-1(HO-1)或一氧化碳(CO)來抑制細菌脂多糖體所誘導iNOS的表現。這些發現不但有助於進一步了解丹參的抗發炎機制，也提供將來臨床實用的根據，可提供參與的臨床醫師未來從事 in vivo 研究甚至臨床研究的基礎。

五.參考文獻

Choi, H. S., Cho, D. I., Choi, H. K., Im, S. Y., Ryu, S. Y., and Kim, K. M. 2004. Molecular mechanisms of inhibitory activities of tanshinones on lipopolysaccharide-induced nitric oxide generation in RAW 264.7 cells. Arch Pharm Res 27, 1233-1237.

Dittmann, K., Gerhauser, C., Klimo, K., and Hamburger, M. 2004. HPLC-based activity

profiling of Salvia miltiorrhiza for MAO A and iNOS inhibitory activities. Planta Med 70, 909-913.

Jang, S. I., Jeong, S. I., Kim, K. J., Kim, H. J., Yu, H. H., Park, R., Kim, H. M., and You, Y. O. 2003. Tanshinone IIA from Salvia miltiorrhiza inhibits inducible nitric oxide synthase expression and production of TNF-alpha, IL-1beta and IL-6 in activated RAW 264.7 cells. Planta Med 69, 1057-1059.

Lee, T. S., Tsai, H. L., and Chau, L. Y. 2003. Induction of heme oxygenase-1 expression in murine macrophages is essential for the anti-inflammatory effect of low dose 15-deoxy-Delta 12,14-prostaglandin J2. J Biol Chem 278, 19325-19330.

Lin, C. H., Wu, C. H., Thum, W. Y., Ho, Y. S., and Lee, H. M. 2002. Involvement of p38 mitogen-activated protein kinase in PLL-AGE-induced cyclooxgenase-2 expression. Eur J Pharmacol 438, 143-152.

Martin, D., Rojo, A. I., Salinas, M., Diaz, R., Gallardo, G., Alam, J., De Galarreta, C. M., and Cuadrado, A. 2004. Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. J Biol Chem 279, 8919-8929.

Nguyen, T., Sherratt, P. J., and Pickett, C. B. 2003. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. Annu Rev Pharmacol Toxicol 43, 233-260.

Otterbein, L. E., and Choi, A. M. 2000. Heme oxygenase: colors of defense against cellular stress. Am J Physiol Lung Cell Mol Physiol 279, L1029-1037.

Wang, X., Wei, Y., Yuan, S., Liu, G., Lu, Y., Zhang, J., and Wang, W. 2005. Potential anticancer activity of tanshinone IIA against human breast cancer. Int J Cancer 116,

799-807.

Zhou, G., Jiang, W., Zhao, Y., Ma, G., Xin, W., Yin, J., and Zhao, B. 2003. Sodium tanshinone IIA sulfonate mediates electron transfer reaction in rat heart mitochondria. Biochem Pharmacol 65, 51-57.