牛樟芝萃取物抑制微小膠質細胞發炎機制之探討

Study on the anti-inflammatory mechanisms of Taiwanofungus in microglia

中文摘要

樟芝 (Taiwanofungus，學名 Taiwanofungus camphoratus)爲台灣特有的真菌類，具有抗發炎、抗氧化、血管舒張以及抗 B 型肝炎病毒等生理活性，然而，其詳細抗發炎成分與機制尚未明確。在本研究論文中，我們使用野生型樟芝、液態培養樟芝和固態培養樟芝的冷水、甲醇和熱水三種萃取物，並檢驗它們在微小膠質細胞中的抗發炎作用，並進而探討樟芝萃取物對於預防腦部退化性疾病的產生可能扮演的角色。微小膠質細胞爲腦中的免疫細胞，扮演著監控與防禦的角色。然而活化的微小膠質細胞會大量表現誘生型一氧化氮合成? (inducible nitric oxide synthase, iNOS)並使 iNOS 的代謝物一氧化氮 (NO) 大量產生，iNOS 和 NO 的生合成對於發炎反應和腦部退化性疾病的形成扮演重要的角色。首先，以不同濃度及種類的樟芝萃取物處理 EOC13.31 微小膠質細胞，再以脂多醣 (LPS)和干擾素- (IFN-)誘導 EOC13.31 微小膠質細胞發炎，並偵測 iNOS 的表現。西方點墨法及反轉錄-聚合?連鎖反應顯示，在 EOC13.31 微小膠質細胞內，野生型樟芝、液態培養樟芝和固態培養樟芝的冷水、甲醇和熱水萃取物皆可抑制 LPS 和 IFN- 所誘導的 iNOS 蛋白質和 mRNA 表現;然而，在我們所測試的九種樟芝萃取物中，野生型樟芝甲醇萃取物抑制效果最明顯，且隨著濃度增加其抑制 iNOS 和 TNF- 表現的效果越顯著。另外，我們進一步證實由野生型樟芝甲醇萃取物中分離出的 CKJ-35-2 抑制 LPS 和 IFN- 所誘導的 iNOS 蛋白質表現的效果最顯著。爲了確認其中的抑制機轉，西方點墨法之結果顯示野生型樟芝甲醇萃取物可抑制 EOC13.31 微小膠質細胞內 LPS 和 IFN- 所誘導 p-STAT1 (signal transducer and activator of transcription-1)、p-ERK (extracellular signal-regulated protein kinases)、p-JNK (c-Jun NH2-terminal protein kinases)的活性，除此之外野生型樟芝甲醇萃取物也可抑制 I B (inhibitor B)的降解和 p-I B 的活性。當我們改以 -Amyloid (25 M)刺激細胞發炎時，野生型樟芝甲醇萃取物同樣隨濃度增加而抑制 iNOS 表現的效果越顯著。綜合以上結果，證實樟芝甲醇萃取物爲一種抗發炎物質，可有效抑制腦部微小膠質細胞中 iNOS 和 TNF- 的表現，進而抑制微小膠質細胞發炎的情形。此篇研究報告結果顯示樟芝萃取物調節 iNOS 的特性也許在預防腦部細胞的發炎反應及腦部退化性疾病的形成扮演重要的角色。

英文摘要

Taiwanofungus (Taiwanofungus camphoratus), a medicinal mushroom in Taiwan, is

reported to provide several therapeutic benefits including anti-inflammation, antioxidation, vasorelaxation, antihepatitis B surface antigen activities, but the underlying molecular mechanisms are not well understood. In this study, we used three cultured types of wild type, Liquid-state culture, and solid-state culture Taiwanofungus extracts, and to examine their anti-inflammatory effect in microglia cells and the possible role in the protection of neurodegenerative diseases. Since microglia is the immune cell in the brain, it plays a defense and monitoring role. However, activated microglia expresses high level of inducible nitric oxide synthase (iNOS) and its metabolite nitric oxide (NO) that significantly contributes to the pathogenesis of neurodegenerative diseases. First, EOC13.31 microglia was treated with various kinds of Taiwanofungus extracts and lipopolysaccharide (LPS) and interferon- (IFN-), then detected the iNOS expression. Western blot and RT-PCR analysis demonstrated that cold water, methanol, and hot water extracts of wild type, Liquid-state culture, and solid-state culture Taiwanofungus significantly blocked the protein and mRNA expression of iNOS in LPS and IFN- -activated microglia. Among nine extracts of Taiwanofungus, methanol extracts of wild type Taiwanofungus was the most potent inhibitor on the iNOS and TNF- expression, and the inhibition by a dose-dependant manner. The CKJ-35-2 fraction of methanol extracts of wild type Taiwanofungus, effectively inhibited the iNOS expression. To clarify the mechanisms involved, methanol extracts of wild type Taiwanofungus was found to inhibit the LPS and IFN- -induced the phosphorylation of extracellular signal-regulated protein kinases (ERK), c-Jun NH2-terminal protein kinases (JNK) and signal transducer and activator of transcription-1 (STAT1). Moreover, methanol extracts of wild type Taiwanofungus also inhibited NF- B activition through the prevention of inhibitor B (I B) degradation and phosphorylation. These results suggests that modulation of iNOS expression by Taiwanofungus extracts may be important in the prevention of inflammation and neurodegenerative diseases.