骨碎補對骨母細胞生長作用的影響

Effect of Drynaria fortunei on Osteoblast Cells Growth

中文摘要

第一部分：骨碎補的成分分離

槲蕨（Drynaria fortunei（KUNTZE）J. SMITH）爲傳統常用之治療骨科疾病的藥材，具有補腎活血、壯筋續骨的功效，爲水龍骨科（Polypopdiaceae）槲蕨屬（Drynaria（BORY）J.SMITH）的植物。本實驗主要爲分離純化槲蕨的成分，利用各種不同的管柱充填劑如：Sephadex LH-20、ODS、Diaion 與 MCI-gel 的逆相管柱層析法，再以階梯濃度的水-甲醇溶媒系統作爲移動相沖提，所分離純化出之化合物以其氫、碳核磁共振光譜、質譜與紅外線光譜來判斷其結構，並與文獻比對後，鑑定爲：(-)-epicatechin-3- O-b-D-allopyranoside。

第二部分：骨碎補成分對骨母細胞生長作用的影響

骨母細胞爲骨生成的重要因子，並且與許多骨科疾病有關，例如：骨質疏鬆症、骨折等，本實驗即是將槲蕨的已知成分柚皮甙(narigin)與經純化分離出之(-)-epicatechin-3-O-b-D-allopyranoside，於初代骨母細胞的培養模式中，再藉由各種生化分析之表現，如細胞活性測試（MTT assay)、鹼性磷酸酵素、乳糖脫氫酵素、蛋白質量測定與鈣染色法等來評估其影響。就細胞活性測試（MTT assay）而言，當加入(-)-epicatechin-3-O-b-D-allopyranoside (10ug/ml)或柚皮甙(1ug/ml)培養第七天時呈現明顯的細胞量增加（$p<0.05$）；就總蛋白質測定量來判定，亦有明顯的增加（$p<0.05$）；但就酵素表現而言，當加入(-)-epicatechin-3-O-b-D-allopyranoside 或柚皮甙時，對於每單位鹼性磷酸酵素並沒有明顯的差異，乳糖脫氫酵素亦是如此，而鈣染色上也沒有大量增加的情形。由以上的結果可以顯示(-)-epicatechin-3-O-b-D-allopyranoside 或柚皮甙對於骨母細胞的增長有正向的幫助，但可能還需更多的實驗加以驗證。

英文摘要

Part I. Isolation of Drynaria fortunei (KUNTZE) J. SMITH Components

The rhizoma of Drynaria fortunei (KUNTZE) J. SMITH (polypodiaceae) used in the traditional Chinese medicine,treat for invigorating kidney, increasing blood circulation, strengthening connective tissue and restoring bones.

In this study, the constituents of the plant were isolated, purified by using a combination of column chromatography on sephadex LH-20, ODS, Diaion and MCI-gel, and gradient concentration of water-methanol solvents system achieve to purify the compound. Based on the spectra data of 1H-NMR, 13C-NMR, NOESY, MS, and IR, and comparison with those in the literatures, the compound was identified as: (-)-epicatechin-3-O-b-D-allopyranoside.

Part II. Effect of Drynaria fortunei (KUNTZE) J. SMITH on Osteoblast Cells Growth

Osteoblast cells play an impartment role in bone formation, and related to several diseases such as osteoporosis and bone fracture. In the present study, primary cultures of osteoblasts were used to investigate the effect of (-)-epicatechin-3-O-b-D-allopyranoside and Naringin isolated from Drynaria fortunei by several biochemical analysis: cell viability test (MTT), alkaline phosphatase(ALP), micro protein determination, lactate dehydrogenase (LDH), and Von Kossa stain.

A significant ($P<0.05$) increase of 20% in MTT assay and micro protein determination were observed in osteoblasts treated with (-)-epicatechin- 3-O-b-D-allopyranoside or narinign when compared to the control group at 7 days, but osteoblast treated with (-)-epicatechin-3-O-b-D- allopyranoside or narinign didn’t showed significantly in ALP and LDH expression compared to control. It suggested that these compounds may have potential to be an adjuvant on proliferation of osteoblast cells, but need more to proof.