骨碎補之類黃鹼素對蝕骨細胞活性的影響

Effect of Flavonoids Isolated from Drynaria fortunei on Osteoclasts Activity

中文摘要

骨碎補（Gusuibu）爲水龍骨科（Polypodiaceae）槲蕨（Drynaria fortunei (Kunze) J. Sm.）之乾燥根莖，根據過去許多的文獻記載，骨碎補爲具有預防及治療骨相關疾病的傳統中藥。而根據本實驗室逐年建立之研究模式，骨碎補經活性分析證實對骨母細胞有促進之作用，而其分離之成分(-)-epicatechin-3-O-β-D-allopyranoside，也已於本實驗室證明對骨母細胞，有促進增生的作用 (簡尙志，2002)。本實驗再對骨碎補於蝕骨細胞，與骨質的增生和再吸收作用過程，對其活性機轉作更深入之探討。

本實驗用 Wistar 新生老鼠做細胞初代培養，以體外試驗模式探討骨碎補對蝕骨細胞的影響，經由不同時間點的細胞存活率測試（MTT assay），分析酸性磷酸酵素（ACP）和乳酸去氫酵素（LDH）的含量來測量酵素活性。並佐以酒石酸磷酸酵素染色（TRAP stain）觀察蝕骨細胞分化成熟過程的影響；用 2'',7''-Dichlorofluorescein（DCF assay）測試蝕骨細胞內活性氧物種；抽取細胞 DNA 以電泳分析，研究蝕骨細胞凋亡（apoptosis）的表現。另外抽取蝕骨細胞中 RNA，以同步定量聚合酶連鎖反應方法（real-time PCR），以 TaqMan® probe 檢視蝕骨細胞 osteoprotegerin（OPG）和 RANK-ligand（RANKL）的基因表現，是否具抑制蝕骨細胞活性的作用。

本實驗將從槲蕨根莖經純化分離出之(-)-epicatechin-3-O-β-D-allopyranoside，已於本實驗室證明對骨母細胞有促進增生的作用。就蝕骨細胞活性測試（MTT assay）而言，當加入(-)-epicatechin-3-O-β-D-allopyranoside (10 μg/mL)培養第七天時即呈現明顯的細胞抑制（$p<0.05$），且明顯抑制酸性磷酸酵素活性，這情形也表現在抗酒石酸磷酸酵素染色上。從細胞內的氧化壓力分析，(-)-epicatechin-3-O-β-D-allopyranoside 促使活性氧物種濃度上升 10%，而在細胞 apoptosis 表現上，實驗組和控制組間，發現並未導致蝕骨細胞有 apoptosis 的趨向。在 RNA 表現的方面，(-)-epicatechin-3-O-β-D-allopyranoside 讓 OPG 表現增加並抑制了 RANKL，所以有抑制蝕骨細胞的活性。

由以上的結果可以顯示(-)-epicatechin-3-O-β-D-allopyranoside 對於骨質之再吸收有抑制的效果，其在抑制蝕骨細胞成熟和分化，並且降低成熟蝕骨細胞中酸性磷酸酵素的活性，而非經由細胞凋亡的機轉。

英文摘要

The traditional Chinese medicine: Gusuibu [Drynaria fortunei (Kunze) J. Sm.] (Polypodiaceae) was commonly used to manage disorders of orthopedics and has

been claimed to have therapeutic effects on bone healing. In the preliminary study, the Gusuibu and its isolated pure compound: (-)-epicatechin-3-O-β-D-allopyranoside has been elucidated to be capable to enhance the proliferation of osteoblasts. In this study, we tried to further investigate the molecular mechanism of gusuibu on the bone cells metabolism.

Primary culture of osteoclasts cells were isolated from newborn Wistar rats. The effects of Drynaria fortunei on bone cell viability were determined by the method of MTT assay. The effects on osteoclasts cells activities were analyzed by acid phosphatase (ACP) and lactate dehydrogenase (LDH). The differentiation of osteoclasts was analyzed by tartrate-resistant acid phosphatase stain. The quantification of intracellular reactive oxygen species (ROS) was assayed by 2'',7''-Dichlorofluorescein (DCF) method. Moreover, the RNA extracted from osteoclasts cells were applied on the real-time PCR, and then determined by TagMan® probe. The gene expression of OPG (osteoprotegerin) and RANKL (RANK-ligand) could further tell if they have negative influence on activity of osteoclast cells.

The results showed that the (-)-epicatechin-3-O-β-D-allopyranoside [extracted from the rhizoma of Drynaria fortunei (KUNTZE) J. SMITH (Polypodiaceae)] has an adjuvant on proliferation of osteoblast cells. The 10 μg/mL (-)-epicatechin-3-O-β-D-allopyranoside inhibits the activity of osteoclasts during proliferation stage as manifested in the MTT assay, enzyme activity of ACP and TRAP stain. In the presence of (-)-epicatechin-3-O-β-D-allopyranoside, the intracellular reactive oxygen species level of osteoclasts increased. On the concept of RNA expression, (-)-epicatechin-3-O-β-D-allopyranoside improved the expression of OPG but meanwhile inhibited RANKL. It showed the inhibition of osteoclasts cells activity.

The potential of (-)-epicatechin-3-O-β-D-allopyranoside on bone resorption was down-regulator on osteoclastic activity and inhibited the acid phosphatase activities but not induced the apoptosis of osteoclasts.