雙磷酸鹽類藥物對於 MC3T3-E1 細胞增生及分化之調控作用

Regulatory Effect of Bisphosphonates on Proliferation and Differentiation of MC3T3-E1 Cells

中文摘要

雙磷酸鹽類藥物對於骨質疏鬆症及其併發症的預防，限制惡性腫瘤的骨轉移具有相當明確的臨床療效，然而長期使用所衍生的骨代謝速率(Bone turnover rate)降低，骨癒合不佳，甚至是顎骨壞死的問題，近年來卻逐漸浮現，也引起醫界及牙醫界的高度重視；目前對於這一類藥物的藥理機轉了解相當有限，甚至對於骨骼調控系統的實際作用依舊存在非常多的爭議。因此本實驗藉由膜內骨生成前類骨母細胞(MC3T3-E1 mouse pre-osteoblastic cells)爲實驗模型，利用 Cell Proliferation Reagent WST-1 assay 測定 MC3T3-E1 在高濃度(10-6M)及低濃度(10-8 M) Alendronate 藥物刺激下的增殖(proliferation)情形，並且使用 RT-qPCR 檢測分化轉錄因子(differentiation transcription factors): Runx-2，Osterix，及 Osteocalcin；此外我們也觀察雙磷酸鹽藥物是否會藉由改變 MC3T3-E1 細胞的 OPG/RANKL system，進而抑制蝕骨細胞功能，降低骨代謝速率。統整我們的實驗結果，發現雙磷酸鹽類藥對於造骨細胞分化初期的轉錄因子 Runx-2 和 Osterix 會產生抑制的作用，且藥物濃度愈高則抑制效果愈明顯(Correlation is significant at $p<0.01$ level)，其中高濃度(10-6M)對 Runx-2 抑制現象在加藥後 24 小時非常強烈(Mann-Whitney U Test，$p<0.05$)，此外我們以 ELISA 測量上清液中終端產物 Osteocalcin 濃度，在藥物作用下出現濃度降低的現象，雖未能有統計上顯著的差異，經由實驗結果我們發現在 10-6 M 至 10-8M 濃度區間的雙磷酸鹽類藥物雖不會抑制前類骨母細胞的增殖(proliferation)，但對分化作用(differentiation)有明顯的抑制現象。此外不論利用 RT-qPCR 或 ELISA 都無法測量 MC3T3-E1 的 RANKL 基因表現，原因應該是缺少適當的培養條件(如:加入 1,25-(OH)2D3)，但是 OPG 的基因及上清液(supernatant)中濃度與胞的生長及分泌物累積有關，而不受雙磷酸鹽類藥物抑制。從我們的實驗結果發現雙磷酸鹽藥物可能改變 MC3T3-E1 細胞的分化，但是否同時因此調控蝕骨細胞的活性和骨骼生理代謝速率則需要進一步研究。

英文摘要

Medication with bisphosphonates has definitive clinical results to treatment of osteoporosis, prevention of its complications, and inhibition of bone metastasis associated with malignant tumor. In recent years, its long term effects such as decrease in bone turnover rate, unsatisfying bone healing ability and necrosis of jaw bones has raised concerns in the field of medicine and dentistry. The detailed mechanism and its alteration to the skeletal system have yet to be understood.

In this experiment, we used MC3T3-E1 mouse pre-osteoblastic cells as model. Cell Proliferation Reagent WST-1 assay is used to detect the proliferation after stimulating with different concentrations of Alendronate (10-6M, 10-8 M). RT-qPCT technique is also used to monitor differentiation transcription factors Runx-2, Osterix and Osteocalcin. We would also determine whether bisphosphonate alters the OPG/RANKL system of MC3T3-E1 pre-osteoblastic cells to inhibit osteoclast activities and consequently reduce bone turnover rate.

We observed an inhibitory effect to Runx-2 and Osterix in MC3T3-E1 cell during early stages of differentiation. With higher concentrations of bisphosphonate, the more inhibitory effect is likely to occur (correlation is significant at $p<0.01$ level). Runx-2 is strongly inhibited at the high concentration (10-6M) after 24 hours of drug administration (Mann-Whitney U Test，$p<0.05$). A decrease of Osteocalcin concentration in supernatants was detected using the ELISA technique, even though it was not statistically significant. From our experiment, we discovered that cell proliferation was not inhibited by bisphosphonate in concentrations ranging from 10-6 M to 10-8M, but its differentiation was. We were unable to detect the RANKL gene expression of MC3T3-E1, but OPG gene and the concentration of supernatant is associated with cell growth and accumulation of secretion. We concluded that bisphosphonate can alter the differentiation of MC3T3-E1 cell, but further study is needed to verify its modulation of osteoclastic activity and physiological metabolic rate of bone.