行政院國家科學委員會專題研究計畫成果報告

動情素及環境動情素影響成骨細胞生長之機轉研究

Studies on the mechanism(s) of action of estrogen and environmental estrogens on the cell growth of osteoblast

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一、中文摘要

動情素(estrogen)在生理病理上有其特殊意義，對於骨骼也具有保護作用，但其需透過何種機制來進行則尚未完全清楚。環境動情素(environmental estrogens) 包括有去污劑、殺蟲劑、其他工業化學物如多氯聯苯(hydroxy-PCBs)、2,3,4-TCB、4-OH-alkyl-phenols、多環芳香烴類化合物等，以及緣自植物的 phytosterol esters，已知其中某些物質會與細胞核類固醇受體交互作用而影響到性分化現象。雖然目前對於環境動情素在內分泌及生殖系統的影響有較多的研究，但這些賀爾蒙樣物質對於骨代謝系統的影響也是不容忽視，而有關此方面的研究並不多。在本研究計劃中，我們發現動情素樣物質會促進初級培養成骨細胞(primary culture of rat osteoblast-like cells)及人類成骨細胞株(MG-63 cells)增生，此作用會被 ICI182,780 (動情素拮抗劑)對抗。動情素樣物質亦會促進成骨細胞ERK1/2蛋白磷酸化及誘導二型環氧酵素(cyclooxygenase-2)表現。MAP kinase 抑制劑 PD98059 會對抗動情素樣物質引起的二型環氧酵素及細胞增生表現。這些結果顯示 MAP kinase 系中之 ERK1/2 參與動情素樣物質引起的二型環氧酵素及細胞增生表現過程。

關鍵字：動情素樣物質；骨細胞；細胞生長；二型環氧酵素。

Abstract

It has been found that estrogen had protective effects on bone, but the mechanisms of action have not been fully established. Environmental estrogens include the detergents, pesticide, chlorinated insecticides, polycyclic aromatic hydrocarbons and phytosterol esters, and some of these compounds have recently been reported to modulate sexual differentiation by interacting with nuclear steroid receptors. There is an ongoing scientific debate concerning the potential threat of environmental estrogenic pollutants to animal and human health. So far, the focus has been on endocrinological system and reproductive organs, but environmental estrogens have far more widespread actions like as bone system. Nevertheless, the literatures about the action of these substances on the bone system are rare. In our study, we found that environmental estrogens were capable of increasing the proliferation in primary culture of rat

osteoblast-like cells and human osteoblast-like cell line (MG-63), which could be blocked by estrogen antagonist (ICI182,780). Moreover, these environment estrogens could also trigger the phosphorylation of ERK1/2 and could induce the protein expression of cyclooxygenase-2 (COX-2) in osteoblasts. MAP kinase inhibitor PD98059 was capable of inhibiting the expression of COX-2 and cell proliferation induced by these environmental estrogens. These results indicate that the MAP kinase pathway may be involved in environment estrogens-induced COX-2 expression and cell proliferation in osteoblasts

Keywords: environmental estrogens osteoblasts; cell proliferation; cyclooxygenase-2;

二、緣由與目的

動情素(estrogen)在生理病理上有其特殊意義，對於骨骼也具有保護作用，但其需透過何種機制來進行則尚未完全清楚。環境動情素(environmental estrogens) 包括有去污劑、殺蟲劑、其他工業化學物如多氯聯苯(hydroxy-PCBs)、2,3,4-TCB、4-OH-alkyl-phenols、多環芳香烴類化合物等，以及緣自植物的 phytosterol esters，已知其中某些物質會與細胞核類固醇受體交互作用而影響到性分化現象。環境動情素對於動物與人類健康的威脅，近年來受到科學家們的重視。環境動情素樣污染物可以藉著其動情素樣性質而對內分泌及生殖系統產生影響，且可能與某些癌症之發展有關。抽煙的停經後老婦人相較於未抽煙者有較低骨密度(Law et al.,1997)；而香煙中已知含有環境動情素樣污染物（例如多環芳香烴類化合物）。雖然目前對於環境動情素在內分泌及生殖系統的影響有較多的研究，但這些賀爾蒙樣物質對於骨代謝系統的影響也是不容忽視，而有關此方面的研究並不多。在嚙齒類及人類骨細胞已被證實可表現及存在動情素受體(estrogen receptor)，而動情素和環境動情素調節骨細胞生長的作用也是需透過此受體再將反應訊息傳遞下去。動情素在體外細胞研究模式上被發現會調節成骨細胞(osteoblast)的生長，及影響副甲狀腺激素對成骨細胞的調節功能，動情素的這些作用需透過動情素受體，且胰島素樣生長因子−1 (insulin-like growth factor-1)可能參與其中反應(Nasu et al.,2000)。動情素對於骨骼的保護作用可能可經由一氧化氮(NO)媒介，因有實驗證實一氧化氮生成酵素(NO synthase)抑制劑會抵消動情素對於去卵巢(ovariectomized)實驗動物骨骼傷害的保護作用，且動情素亦具有刺激成骨細胞 type III NOS (eNOS)活性表現的能力(Armour and Ralston, 1998)。這些研究對於骨細胞代謝的瞭解非常重要。

目前有關動情素和環境動情素對於成骨細胞生長調節的真正機轉仍然未能完全明白。本研究計劃的目的是在於探討動情素及環境動情素對於成骨細胞生長的影響及可能訊息傳遞機轉。本計劃使用大鼠的初級培養成骨細胞(primary culture of rat osteoblast-like

cells)及人類成骨細胞株(MG-63 cells)為研究對象，利用藥理學及細胞或分子生物學技術來進行實驗，主要目的在深入探討植物動情素及環境動情素對於成骨細胞生長的影響及其可能訊息傳遞過程。

三、結果與討論

在本研究計劃中，我們發現動情素樣物質(包括環境動情素及植物動情素)會促進初級培養成骨細胞 (primary culture of rat osteoblast-like cells)及人類成骨細胞株(MG-63 cells)增生，此作用會被ICI182,780 (動情素拮抗劑)對抗。動情素樣物質亦會促進成骨細胞ERK1/2蛋白磷酸化及誘導二型環氧酵素(cyclooxygenase-2)表現。MAP kinase抑制劑PD98059會對抗動情素樣物質引起的二型環氧酵素及細胞增生表現。這些結果顯示MAP kinase系中之ERK1/2參與動情素樣物質引起的二型環氧酵素及細胞增生表現過程。

四、自評

本研究計劃之執行成果使我們對於植物動情素及環境動情素對成骨細胞生長之影響及可能之訊息傳遞機轉有更進一步的瞭解。我們希望能以此為基礎，而能在未來對於植物動情素及環境動情素的生理病理甚至治療評估上有所助益。

五、參考文獻

Armour KE, Ralston SH (1998) Estrogen upregulates endothelial constitutive nitric oxide synthase expression in human osteoblast-like cells. Endocrinology 139:799-802.

Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 1993;101:378-384.

Damien E, Price JS, Lanyon LE (2000) Mechanical strain stimulates osteoblast proliferation through the estrogen receptor in males as well as females. J. Bone Miner. Res. 15:2169-2177.

Dodge JA, Glasebrook AL, Magee DE, Phillips DL, Sato M, Short LL, Bryant HU (1996) Environmental estrogens: effects on cholesterol lowering and bone in the ovariectomized rat. J. Steroid Biochem. Mol. Biol. 59:155-161.

Guillette L, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR.(1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ. Health. Perspect. 102:680-688.

Law MR, Cheng R, Hackshaw AK, Allaway S, Hale AK (1997) Cigarette smoking, sex hormones and bone density in women. Eur. J. Epidemiol. 13:553-558.

Nasu M, Sugimoto T, Kaji H, Chihara K (2000) Eestrogen modulates osteoblast proliferation and function regulated by parathyroid hormone in osteoblastic SaOS-2 cells: role of insulin-like growth factor (IGF)-I and IGF-binding protein-5. J. Endocrinol. 167:305-313.

Safe SH. (1997) Xenoestrogens and breast cancer. N. Engl. J. Med. 337:1303-1304.

Sonnenschein C, Soto AM. (1998) An updated review of environmental

estrogen and androgen mimics and antagonists. J.Steroid Biochem. Molec. Biol. 65:143-150.

Vidal O, Kindblom LG, Ohlsson C (1999) Expression and localization of estrogen receptor-beta in murine and human bone. J. Bone Miner. Res. 14:923-929.

White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. (1994) Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135:175-182.