The Use of Simvastatin with Aromasin in An Ovariectomized Rat Model: Effects on the Skeletal System

Sung-Hsiung Chen, MD; Fong-Fu Chou[1], MD; Jih-Yang Ko, MD

Background: Many studies have reported the positive effect on bones of statins that inhibit the action of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and suppress hepatic cholesterol biosynthesis. Recent data suggest that statins used in the treatment of hypercholesterolemia decrease fracture risk and increase bone mineral density (BMD). Aromasin (an aromatase inhibitor) is an effective and well-tolerated drug used in endocrine therapy for the treatment of hormone-sensitive early breast cancer in postmenopausal patients. It has a catabolic effect on the skeletal system and can therefore significantly increase the incidence of fractures. Our study aims to determine the effects of Aromasin and simvastatin plus Aromasin on the BMD in an ovariectomized rat model.

Methods: In total, 27 female Sprague Dawley rats were subjected to a bilateral oophorectomy. One month after the oophorectomy, the rats were divided into the following 3 groups: (1) The control group, in which water was administered; (2) the Aromasin group in which Aromasin was administered orally; and (3) the Aromasin plus simvastatin group in which a combination of Aromasin and simvastatin was administered orally. The BMD of the lumbar spine (L1–L5) and left femoral bone was measured using dual-energy X-ray absorptiometry (DXA) 1 month after the ovariectomy and 3 months after treatment began. Blood was drawn at the time of oophorectomy and 3 months after treatment began to check the levels of calcium, phosphorus, and alkaline phosphatase (alk-ptase).

Results: In the Aromasin plus simvastatin group, the BMD of both the lumbar spine ($p = 0.003$) and the left femoral bone ($p = 0.001$) increased significantly after 3 months of treatment. In comparison with the Aromasin group, the Aromasin plus simvastatin group showed a significant increase in the BMD of both the lumbar spine and the left femoral bone ($p = 0.04$ and $p = 0.005$ respectively). In the Aromasin group, the BMD of the left femoral bone ($p = 0.01$) and that of the lumbar spine both decreased significantly ($p = 0.001$). The calcium, phosphorus, and alk-ptase levels were not significantly different among the 3 groups.

Conclusions: In the Aromasin group, catabolic effects on the skeletal system were observed. In the Aromasin plus simvastatin group, the BMD significantly increased. Thus statins may have therapeutic application in the treatment of osteoporosis using Aromasin since they can counterbalance the adverse effects of this drug.
(Chang Gung Med J 2010;33:509-14)

Key words: bone mineral density, simvastatin, Aromasin

From the Department of Orthopaedic Surgery; [1]Department of General Surgery, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan.
Received: May 15, 2009; Accepted: Dec. 7, 2009
Correspondence to: Dr. Fong-Fu Chou, Department of General Surgery, Chang Gung Memorial Hospital-Kaohsiung Medical Center. 123, Dapi Rd., Niaosong Township, Kaohsiung County 833, Taiwan (R.O.C.) Tel.: 886-7-7317123 ext. 8093;
Fax: 886-7-7354309; E-mail: choulu@ms4.hinet.net

Aromasin is a steroidal aromatase inactivator that acts to reduce the incidence of breast cancer by lowering the tissue levels of estrogen,[1,2] It is currently licensed for the management of advanced breast cancer in postmenopausal women.[3] Plasma levels of estradiol have been shown to fall in women being treated with aromatase inhibitors for breast cancer. However, the potential acceleration of osteoporosis with an increase in the incidence of fractures needs to be carefully assessed.[2,4]

Statins (3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors) stimulate bone formation in vitro and in rodents and are used for the treatment of hypercholesterolemia.[5] Several studies have demonstrated the beneficial effect of statins on bone mineral density (BMD) and fracture risk.[6,7] Simvastatin has been shown to stimulate growth factors that act on bones and increase bone formation in experimental models.[5,8] There have been no previous studies on the protective effect of statins on bones when used in combination with aromatase inhibitors. The purpose of this study was to determine the effects of Aromasin and a combination of simvastatin and Aromasin on BMD in sexually mature, ovariectomized rats.

METHODS

Preparation of the animals

Twenty-seven female Sprague-Dawley rats that were approximately 12 weeks of age and weighed 300 g (range: 280~320 g), were selected for this study. All rats were subjected to bilateral oophorectomy.[9] One month after the ovariectomy, the rats were divided into the following 3 groups: (1) the control group; (2) Aromasin group; and (3) Aromasin plus simvastatin group. The body weight of each rat was measured at baseline and 3 months after the ovariectomy. The procedure was approved by The Animal Use and Care Committee.

Drug administration

In the control group, water was administered using a pill gun. In the Aromasin group, Aromasin, 1 mg/kg/ 5 times per week was administered orally 1 month after the oophorectomy. In the simvastatin and Aromasin group, simvastatin (approximately 6.5 mg/kg/ 5 times per week) was administered orally in addition to Aromasin. The drugs were administered for a total duration of 12 weeks. This drug regimen has been previously demonstrated to be pharmacologically active and to not exert toxic effects in these animals.[10,11]

Dual-energy-X-ray absorptiometry

Dual-energy-X-ray absorptiometry (DXA) was used to evaluate the potential effects of drug treatment on the density of the lumbar spine (L1-L5) and left femoral bone. BMD (in g/cm^2) was determined using a Delphi A model (Hologic, Bedford, Mass, U.S.A.) at 1 and 4 months after ovariectomy (3 months after the beginning of treatment). Blood was drawn from the rats' tails at the time of oophorectomy and 3 months after treatment began to check the levels of calcium, phosphorus, and alkaline phosphatase (alk-ptase).

Statistical evaluation

Data were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using repeated measures ANOVA (via an analysis of variance [ANOVA] model) to test for significant changes between 1 and 4 months after ovariectomy (3 months after the treatment). A significance level (p value) of 0.05 was used, with a $p \leqq 0.05$ considered a statistically significant change.

RESULTS

The BMD of the left femoral bone 1 month after ovariectomy was 0.4926 $\pm$ 0.0332 g/cm^2 in the control group, 0.4524 $\pm$ 0.024 g/cm^2 in the Aromasin plus simvastatin group, and 0.46084 $\pm$ 0.058 g/cm^2 in the Aromasin group. Three months after treatment, the BMD of the left femoral bone was 0.5537 $\pm$ 0.0304 g/cm^2 in the control group, 0.5940 $\pm$ 0.0221 g/cm^2 in the Aromasin plus simvastatin group, and 0.4100 $\pm$ 0.0469 g/cm^2 in the Aromasin group. The increase in the BMD of the femoral bone was significant in the Aromasin plus simvastatin group (p = 0.001). The decrease in the BMD of the femoral bone was significant in the Aromasin group (p = 0.01) but not in the control group (p = 0.177) (Fig. 1A). The BMD of the lumbar spine (L1–L5) 1 month after ovariectomy was 0.2858 $\pm$ 0.011 g/cm^2 in the control group, 0.3034 $\pm$ 0.019 g/cm^2 in the Aromasin plus simvastatin group, and 0.3318 $\pm$ 0.0056 g/cm^2 in the Aromasin group. Three months

after treatment, the BMD of L1–L5 was 0.3883 ± 0.0259 g/cm^2, 0.3702 ± 0.0095 g/cm^2, and 0.3174 ± 0.0071 g/cm^2 in the control group, Aromasin plus simvastatin group, and Aromasin group, respectively. The increase in BMD was significant in the Aromasin plus simvastatin group (p = 0.003). A decrease in the BMD was significant in the Aromasin group (p = 0.001) (Fig. 1B). Three months after treatment, the BMDs of L1–L5 (p = 0.036) and the left femoral bone (p = 0.008) were significantly higher in the Aromasin plus simvastatin group than in the Aromasin group. The serum calcium, phosphorus, and alk-ptase levels were not significantly different among the 3 groups. The mean body weight of the rats before ovariectomy was 319 ± 14.9 g in the control group, 312 ± 18.9 g in the Aromasin group, and 307 ± 21.7 g in the Aromasin plus simvastatin group; there was no significant difference in body weight among the 3 groups. Four months later, the mean weight of the rats was 490 ± 15.5 g in the control group, 482 ± 31.7 g in the Aromasin group, and 476 ± 33.0 g in the Aromasin plus simvastatin group; the increases in body weight 4 months after the ovariectomy were significant in all 3 groups (all p < 0.001). However, at the 4-month examination, there was no significant differences between the body weights of the 3 groups.

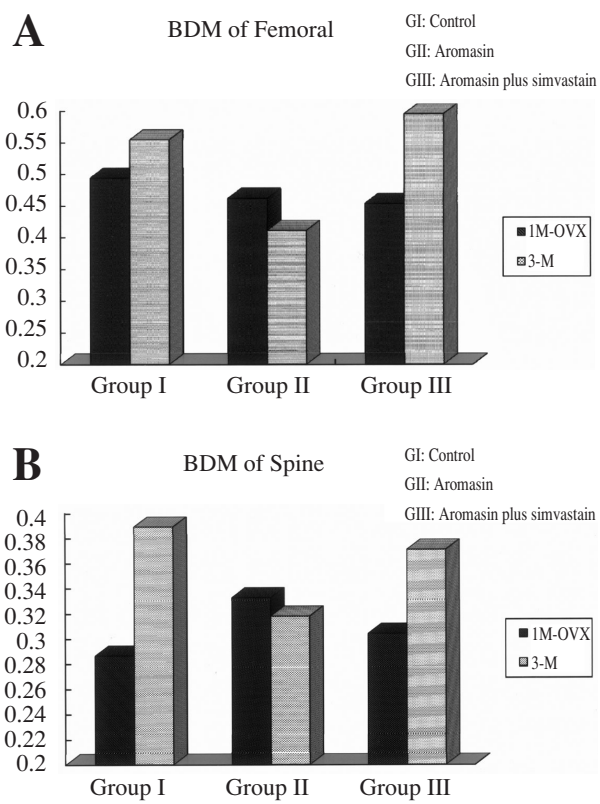

Fig. 1 Comparison of the bone mineral density (BMD) after ovariectomy (1-m OVX) and 3 months (3-M) after the beginning of treatment in the control, Aromasin, and Aromasin plus simvastatin groups. (A) The increase in the BMD of the femoral bone (p = 0.001) was significant in the Aromasin plus simvastatin group. The decrease in the BMD of the femoral bone (p = 0.01) was significant in the Aromasin group but not in the control group (p = 0.177), (B) The increase in BMD of the lumbar spine (p = 0.003) was significant in the Aromasin plus simvastatin group. The decrease in the BMD of the lumbar spine (p = 0.001) was significant in the Aromasin group.

DISCUSSION

There is no doubt that estrogen plays a significant role in breast cancer development. Depriving the tumor of this stimulus is an established method of treating the disease.[12-14] Aromatase inhibitors are a class of compounds that inhibit the synthesis of estrogen from androgen in postmenopausal women. With respect to improving the time to progression, aromatase inhibitors are more effective as first-line treatment for advanced breast cancer than tamoxifen.[13,15] Additionally, this treatment has been shown to reduce the incidence of thromboembolic disease and vaginal bleeding.[15,16] Total suppression of aromatase may lead to adverse side effects such as osteoporosis with an increase in the incidence of fractures.[2,4,17] Osteoporosis is a major cause of morbidity and mortality in postmenopausal women.[18,19]

Treatment of osteoporosis is receiving increasing attention since our population is aging, and the treatment involves altering the balance between bone formation and bone resorption with the goals of influencing the BMD and reducing fracture risk. Many drugs such as parathyroid hormone (PTH), bisphosphonates, and selective estrogen receptor modulators have been investigated extensively. There are some drawbacks. Biophosphates can cause gastroenteric irritation, PTH requires daily injections, and increased BMD is difficult to induce with selective estrogen receptor modulators.[20-22]

Statins have been shown to be competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and are administered orally

in the treatment of hyperlipidemia. Experimental evidence from animal studies has shown that statins promote bone formation as well as increased cancellous bone volume and a corresponding increase in cancellous bone compressive strength.[5,23] Simvastatin has also been shown to increase periosteal bone formation in cortical bone.[24] Many findings suggest that exposure to statins is associated with an increased BMD with a substantially lower risk of developing fracture in humans, even after a short exposure of a few weeks to a few months.[7,25-27]

The use of aromatase inhibitors in postmenopausal women lowers the levels of tissue estradiol. The plasma levels of estradiol decrease in women being treated for breast cancer. This results in potential acceleration of osteoporosis and more seriously, a significant increase in the number of bone fractures. The severity of bone problems can be ameliorated by using drugs that inhibit bone resorption during treatment. There is a clear need for nontoxic and anabolic agents that can be easily applied, such as statins, since they substantially increase bone formation in people who have already suffered substantial bone loss. In our study, the body weight of the rats at 4 months after ovariectomy was significantly higher than that at baseline. However, the differences among the groups were not significant at 4 months after ovariectomy. The effect of body weight on BMD could therefore be ignored.[9]

Decreases in the BMD of the femoral bone and L1–L5 were noted in the Aromasin group. Three months after the start of Aromasin plus simvastatin treatment, the BMD of L1–L5 and the left femoral bone increased significantly. The data suggest that simvastatin may prevent bone loss attributed to the inhibition of ovariectomy-induced bone resorption or Aromasin-induced bone loss. The serum calcium, phosphorus, and alk-ptase levels were not significantly different between the groups since the healthy kidneys in the study groups could excrete calcium and phosphorus effectively to maintain normal levels.

In conclusion, Aromasin has a catabolic effect on the skeletal system. Simvastatin is shown to significantly increase BMD. Thus, simvastatin may have therapeutic application in the treatment of osteoporosis to counterbalance the adverse effects of Aromasin.

REFERENCES

1. Brodie AM, Wing LY. In vitro and in vivo studies with aromatase inhibitor 4-hydroxy-androstenedione. Steroids 1987;50:89-103.
2. Prichard RS, Hill ADK, Dijkstra B, McDermott EW, O'Higgins NJ. The prevention of breast cancer. Br J Surg 2003;90:772-83.
3. Buzdar A, Jonat W, Howell A, Jones SE, Blomqvist C, Vogel CL, Eiermann W, Wolter JM, Azab M, Webster A, Plourde PV. Anastrozole, a potent and selective aromatase inhibitor, versus megestrol acetate in postmenopausal women with advanced breast cancer: results of overview analysis of two phase III trials. J Clin Oncol 1996;14:2000-11.
4. Santen RJ, Yue W, Naftolin F, Mor G, Berstein L. The potential of aromatase inhibitors in breast cancer prevention. Endocr Relat Cancer 1999;6:235-43.
5. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G. Stimulation of bone formation in vitro and in rodents by statins. Science 1999;286:1946-9.
6. Pasco JA, Kotowicz MA, Henry MJ, Sanders KM, Mnutr KS, Nicholson GC. Statin use, bone mineral density, and facture risk: Geelong Osteoporosis Study. Arch Intern Med 2002;162:537-40.
7. Edwards CJ, Hart DJ, Spector TD. Oral statins and increased bone mineral density in postmenopausal women. Lancet 2000;355:2218-9.
8. Maeda T, Matsunuma A, Kawane T, Horiuchi N. Simvastin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. Biochem Biophys Res Commun 2001;280:874-7.
9. Chou FF, Huang SC, Chen SS, Wang PW, Huang PH, Wu KY. Treatment of osteoporosis with TheraCyte-encapsulated parathyroid cells: A study in a rat model. Osteoporos Int 2006;17:936-41.
10. Yee AJM, Bae HW, Friess D, Roth SM, Whyne C, Robbin M, Johnstone B, Yoo JU. The use of simvastatin in rabbit posterolateral lumbar intertransverse process spine fusion. Spine J 2006;6:391-6.
11. Omar WA, Wilson JP, Cox TS. Rhabdomyolysis and HMG-COA reductase inibitors. Ann Pharmacother 2001;35:1096-107.
12. Howell A, Dowsett M. Recent advances in endocrine therapy of breast cancer. BMJ 1997;315:863-6.
13. Baum M, Budzar AU, Cuzick J, Forbes J, Houghton JH, Klijn JG, Sahmoud T. The ATAC Trialist's Group. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. Lancet 2002;359:2131-9.
14. Santen RJ, Harvey HA. Use of aromase inhibitors in

breast carcinoma. Endocr Relat Cancer 1999;6:75-92.
15. Nabholtz JM, Buzdar A, Pollak M, Harwin W, Burton G, Mangalik A, Steinberg M, Webster A, von Euler M. Anastrozole is superior to tamoxfen as first-line therapy for advanced breast cancer in postmenopausal women: results of North American multicenter randomized trial. J Clin Oncol 2000;18:3758-76.
16. Bonneterre J, Buzdar A, Nabholtz JMA, Robertson JF, Thürlimann B, von Euler M, Sahmound T, Webster A, Steinberg M. Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma: results of two randomized trials designed for combined analysis. Cancer 2001;92:2247-58.
17. Love RR. Breast cancer prevention. Oncologist 2002;7:100-2.
18. Grodstein F, Stampfer M. The epideminology for coronary heart disease and estrogen replacement in postmenopausal women. Prog Cardiovasc Dis 1995;38:199-210.
19. Kelloff GJ, Lubet RA, Lieberman R, Eisenhauer K, Steele VE, Crowell JA, Hawk ET, Boone CW, Sigman CC. Aromatase inhibitors as potential cancer chemopreventives. Cancer Epidemiol Biomarkers Prev 1998;7:65-78.
20. Neer RM, Arnaud CD, Zanchettu JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 2001;344:1434-41.
21. Black DM, Cummings SR, Karpf DB, Cauley JA, Thompean DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL, Marcus R, Ott SM, Torner JC, Quandt SA, Reiss TF, Ensrud KE. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fracture. Fracture Intervention Trial Research Group. Lancet 1996;348:1535-41.
22. Siris E, Adachi JD, Lu Y, Fuerst T, Cruns GG, Wong M, Harper KD, Genant HK. Effects of raloxifene on fracture severity in postmenopausal women with osteoporosis: results from the MORE study. Multiple outcomes of raloxifen evaluation. Osteoporos Int 2002;13:907-13.
23. Oxlund H, Andreassen TT. Simvastatin given per orally to adult rats increases the compressive strength of vertebral bodies. J Bone Miner Res 2000;15S1:S549.
24. Oxlund H, Andereassen TT. Simvastatin treatment partially prevents ovariectomy-induced bone loss while increasing cortical bone formation. Bone 2004;34:609-18.
25. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. HMG-CoA reductose inhibitors and the risk of fractures. JAMA 2000;283:3205-10.
26. Chan KA, Ardrade SE, Boles M, Buist DSM, Chase GA, Donahue JG, Goodman MJ, Gurwitz JH, Lacroix AI, Platt R. Inhibitors of hydroxymethyl glutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 2000;355:2185-8.
27. Wang PS, Solomon DH, Mogun H, Avorn J. HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 2000;283:3211-6.

使用 Simvastatin 及 Aromasin 的藥物治療去除卵巢白老鼠的研究模式：骨骼系統的影響

陳松雄 周逢復[1] 郭繼陽

背 景： Statin 類的藥物在臨床可用以降低膽固醇並且發現它們可促進骨骼生長，尤其 simvastatin 有關於促進骨骼生成的報告並不少見，而 Aromasin 臨床上主要用於治療乳癌，但是它有抑制骨骼生成的現象，因此我們利用去除卵巢的白老鼠使用此類藥物來評估它們對於骨質密度的影響如何。

方 法： 在 27 隻白老鼠先做兩側卵巢割除，經過一個月後再隨機分爲三組：(1) 控制組 (2) 使用口服 Aromasin (3) 使用 Aromasin 及 Simvastatin。在割除卵巢的一個月及治療藥物三個月後來測老鼠的腰椎及股骨骨質密度，並且在藥物治療三個月後，抽血檢測鈣、磷及鹼性磷酸鹽。

結 果： 發現控制組的腰椎及股骨骨質密度沒有明顯變化，但是 Aromasin 的腰椎及股骨骨質密度會降低，而使用 Simvastatin 的老鼠其腰椎及股骨骨質密度有明顯的增加，而相較於使用 Aromasin 的老鼠組，有使用 Simvastatin 的老鼠組的腰椎及股骨骨質密度仍有明顯增加。抽血檢測鈣、磷及鹼性磷酸鹽在三組沒有明顯變化。

結 論： Aromasin 會使骨質密度降低而加入 Simvastatin 的藥物可阻止 Aromasin 的作用而使其骨質密度上升。因此，利用此一原理在未來臨床上也許可使用 Simvastatin 來治療因爲使用 Aromasin 所引起的骨質疏鬆症。

(長庚醫誌 2010;33:509-14)

關鍵詞： 骨質密度，simvastatin，Aromasin

長庚醫療財團法人高雄長庚紀念醫院 骨科，[1]一般外科；長庚大學 醫學院
受文日期：民國98年5月15日；接受刊載：民國98年12月7日
通訊作者：周逢復醫師，長庚醫療財團法人高雄長庚紀念醫院 一般外科。高雄縣833鳥松鄉大埤路123號。
Tel: (07)7317123轉8093; Fax: (07)7354309; E-mail: choulu@ms4.hinet.net