Inflammopharmacology 16 (2008) 213–215
0925-4692/08/050213-3
DOI 10.1007/s10787-008-8018-7

Inflammopharmacology

Short Review

Soy isoflavones and their bone protective effects

Y. Zhang[1], W.-F. Chen[2,3], W.-P. Lai[3] and M.-S. Wong[3,*]

[1] Department of Medicine, University of Chicago, Chicago, IL, USA, e-mail: medicineyan@163.com
[2] Department of Physiology, Medical College of Qingdao University, Qingdao 266021, China, e-mail: marychhk@yahoo.com
[3] Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, HKSAR, Hung Hom, Kowloon, Hong Kong SAR, Fax: ++852 23649932, e-mail: bcmswong@polyu.edu.hk

Received 15 May 2008; accepted19 June 2008

Published Online First 26 September 2008

Abstract. Several observational studies have suggested that populations with a high dietary soy intake have a lower incidence of osteoporosis-related fractures when compared to Western populations. However, there has not been consistent data to show that soy isoflavones protect against or lessen bone loss. Studies in our laboratory showed that genistein, the major soy isoflavone, could stimulate osteoblastic functions as well as human breast cancer cell growth. These studies raised the concern of whether it would be safe for women who have a prior history of breast cancer to consume soy isoflavone for management of postmenopausal osteoporosis. As increasing the purity of genistein is known to increase its ability to induce human breast cancer cell growth, current effort in our laboratory is to determine if the *in vivo* bone protective effects will be affected by the complexity of the soy isoflavones extract in ovariectomized mice.

Key words: Soy isoflavones – Genistein – Postmenopausal osteoporosis – Breast cancer – Osteoblastic functions – Bone health

Osteoporosis is a metabolic condition characterized by low bone mass, deterioration of bone tissues and increased risk of fracture (Cooper et al*.,* 2006). It is a worldwide public health problem that creates significant economic burden on society as well as the families of patients who are suffering from related fractures (Cooper et al., 2006; Dennison et al., 2005; Cummings and Melton, 2002). Hormone replacement therapy (HRT) used to be the major regimen for prevention and treatment of postmenopausal osteoporosis. However, with the recent discovery that HRT is associated with an increased risk in postmenopausal women to develop breast, endometrial and ovarian cancers (Davison and Davis, 2003; Nelson et al., 2002), there is a strong demand for developing alternative approaches for the management of osteoporosis.

Several observational studies have suggested that populations with a high dietary soy intake have a lower incidence of osteoporosis-related fractures when compared to Western populations (Ross et al., 1991; Lauderdale et al., 1997). Two recent reviews have addressed the effects of soy isoflavones on bone health in rats and humans and their potential applications in the prevention and treatment of postmenopausal osteoporosis (Atmaca et al., 2008; Weaver and Cheong, 2005). However, the data for supporting the use of soy isoflavones for protection against or reduction of bone loss are inconsistent (Atmaca et al., 2008; Weaver and Cheong, 2005; Geller and Studee, 2006). For example, a recent study by the PHYTOS investigators reported that long-term consumption of isoflavone-enriched foods did not affect bone mineral density (BMD), bone metabolism, as well as hormonal status, in early postmenopausal women from three European countries (Netherlands, Italy and France) (Brink et al., 2008); while another European research group from Italy reported that twenty-four months of treatment with genistein, the major soy isoflavone, had positive effects on BMD in osteopenic postmenopausal women (Marini et al., 2007). In both cases, the conclusions were based on well-designed randomized, double-blind, placebo-controlled trials. Studies by research teams in Hong Kong also reported that the positive effects of soy isoflavone administration (Chen et al., 2003) or high dietary phytoestrogen intake (Mei et al., 2001) were found in postmenopausal women with lower bone mass and not in pre-menopausal women, suggesting that the effects of soy isoflavones on bone health are influenced by hormonal status as well as bone mass status.

The differences in results between observational studies and intervention studies could be attributed to differences

* *Corresponding author*

in the populations being studied as well as the types of soy preparation being employed. Most of the observational studies were carried out in Asian populations in whom soy-based foods were consumed as a normal part of the diet. For example, the soy food listed in food frequency questionnaire for studying relationship between soy food consumption and serum isoflavone in women living in Shanghai, People's Republic of China, included fermented bean curd, soybean milk, fresh bean curd, fried bean curd puff as well as soybeans (Frankenfeld et al., 2004) . The soy preparations used in human intervention studies were quite different from those in the food frequency table, including soy protein powder, soy protein food, isoflavone concentrate or extract, isoflavone-rich soy, genistein, as well as soy foods (Williamson and Manach, 2005). In addition, different dosages of soy isoflavone, duration of treatment, different hormonal status and age of subjects were found in different intervention studies. Most importantly, dietary control in long-term human interventional studies, that is required for detecting significant changes in bone mass, is often difficult to achieve.

Studies in our laboratory have focused on elucidation of mechanism of actions of genistein by using different *in vitro* and *in vivo* models. Our studies showed that genistein could stimulate oestrogen receptor-dependent alkaline phosphatase (ALP) activities, a major osteoblastic cell differentiation marker, in human osteoblastic-like SaOS-2 cells in a concentration-dependent manner (Chen and Wong, 2006). Genistein could also increase osteoprotegrin (OPG) protein expression as well as suppress the expression of receptor activator of NF-κB (RANKL) in SaOS-2 cells, suggesting that it might modulate the process of osteoclastogenesis through direct actions on osteoblastic cells. Our results also indicated that it could modulate the effects of parathyroid hormone (PTH) on bone cells. PTH (10nM) stimulated osteoblastic differentiation, induced RANKL mRNA expression, and suppressed OPG mRNA expression in osteoblastic cells, suggesting that it might stimulate bone formation and bone resorption simultaneously. Pre-treatment of SaOS-2 cells with genistein not only enhanced PTH-induced ALP activity, but also attenuated the induction of RANKL mRNA expression and the suppression of OPG mRNA expression by PTH. These results suggested that genistein could enhance bone formation through modulating the actions of PTH on osteoblastic functions.

Other than its anabolic effects in bone cells, our laboratory has also studied the effects of genistein on human breast cancer (MCF-7) cells (Chen et al., 2003; Chen and Wong, 2004; Chen et al., 2007). Our results indicated that genistein at high concentration (50 to 100 μM) inhibited human breast cancer (MCF-7) cell growth. Its inhibitory action on cell growth was at least in part mediated by the inhibition of estrogen receptor (ER) and serum response factor (SRF) expression transcriptionally and post-transcriptionally (Chen et al., 2003). On the other hand, physiological concentration of genistein (1 μM) mimicked the effect of oestrogen in stimulation of human breast cancer (MCF-7) cell growth (Chen and Wong, 2004; Chen et al., 2007). Our results showed that 1 μM of genistein stimulated the growth of MCF-7 cells by increasing insulin-like growth factor I (IGF-I) receptor and insulin response substrate (IRS-1) expression, by enhancing the signalling pathways that mediated the actions of IGF-I as well as by activating the ER dependent signalling pathways. These results confirmed with the *in vivo* studies by Hsieh et al. (1998), in which the growth of MCF-7 cells in ovariectomized athymic mice increased in response to treatment with diets containing genistein. In addition, they reported that the degree of processing of the soy flour affected the ability of a constant amount of genistein in the diet to induce human breast cancer growth in the ovariectomized athymic mice model (Allred et al., 2004) These studies raised the concern of whether it would be safe for women who have a prior history of breast cancer to consume soy isoflavone for the management of postmenopausal osteoporosis.

To determine if the protective effects of soy isoflavones on bone will be affected by the complexity of the soy extract, the effects of a crude extract of soy (Novasoy®) as well as purified genistein on trabecular bone mineral density (BMD) and bone microarchitecture in ovariectomized (OVX) C57BL/6J mice were compared. Both preparations were added to a phytoestrogen-free diet to provide approximately 500 ppm of genistein (aglycone equivalents). Our results showed that both preparations did not increase uterus weight in OVX mice. Novosoy, but not purified genistein, significantly increased trabecular BMD and decreased trabecular separation in proximal tibia of OVX mice ($P < 0.05$ vs.vehicle-treated OVX mice). The results of this study indicated tht Novosoy was more effective than purified genistein in improving tibial trabecular bone mineral density and bone microarchitecture in ovariectomized mice.

Taken together, the results in our laboratory showed that genistein could stimulate osteoblastic functions in a dose-dependent manner and positively modulated the effects of PTH in human osteoblastic cells. Most importantly, it appears that diet containing a crude extract of soy is effective in improving bone mineral density and microarchitecture in ovariectomized mice.

Acknowledgement. This work was supported by the Central Research Fund from the Hong Kong Polytechnic University (GU135) as well as Shenzhen-Hong Kong Innovation Circle Funding Scheme (2006), PRC. The authors would like to thank the State Key Laboratory of Chinese Medicine and Molecular Pharmacology for providing the support in carrying out this study.

References

Allred, C. D., Allred. K. F., Ju.,Y. H., Goeppinger, T. S., Doerge, D. R. and Helferich, W. G. (2004). Soy processing influences growth of estrogen-dependent breast cancer tumors. *Carcinogenesis* **25(9)**, 1649–57.

Atmaca, A., Kleerekoper, M., Bayraktar, M. and Kucuk, O. (2008) Soy isoflavones in the management of postmenopausal osteoporosis, *Menopause* **15(4)**, 1–10.

Brink, E., Coxam, V., Robins, S., Wahala, K., Cassidy, A., and Branca, F. (2008). Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. *Am. J. Clin. Nutr.* **87**, 761–70.

Chen, W. F. and Wong, M. S. (2004). Genistein enhances insulin-like growth factor signaling pathway in human breast cancer (MCF-7) cells. *J Clin Endo Metab* **89(5)**, 2351–9.

Chen, W. F. and Wong, M. S. (2006). Genistein modulates the effects of parathyroid hormone in human osteoblastic SaOS-2 cells. *Brit. J. Nutr.* **95**, 1039–47.

Chen, W. F., Gao, Q. G. and Wong, M. S. (2007). Mechanism involved in genistein activation of insulin-like growth factor 1 receptor expression in human breast cancer cells. *Brit J Nutr* **98**, 1120–5.

Chen, W. F., Huang, M. H., Tzang, C. H., Yang, M. and Wong, M. S. (2003). Inhibitory actions of genistein in human breast cancer (MCF-7) cells, *Biochim Biophys Acta.* **1638(2)**, 187–96.

Chen, Y. M., Ho, S., Lam, S. H. *et al.* (2003). Soy isoflavones have a favorable effect on bone loss in Chinese postmenopausal women with lower bone mass: a double-blind, randomized, controlled trial. *J Clin. Endocrinol. Metab.* **88**, 4740–7.

Cooper, C., Westlake, S., Harvey, N., Javaid, K., Dennison, E. and Hanson, M. (2006). Review: developmental origins of osteoporotic fracture, *Osteoporos. Int*. **17**, 337–47.

Cummings, S. R. and Melton, L. J. (2002). Epidemiology and outcomes of osteoporotic fractures, *Lancet* **359**, 1761–67.

Davison, S. and Davis, S. R. (2003). Hormone replacement therapy: current controversies, *Clin Endo* **58**, 249–61.

Dennison, E., Cole, Z., Cooper, C. (2005). Diagnosis and epidemiology of osteoporosis, *Curr. Opin. Rheumatol.* **17**, 456–61.

Frankenfeld, C., Lampe, J. W., Shannon, J. *et al*. (2004). Frequency of soy food consumption and serum isoflavone concentrations among Chinese women in Shanghai, *Public Health Nutr* **7(6)**, 765–72.

Geller, S. E. and Studee, L. (2006). Soy and red clover for mid-life and aging, *Climacteric* **9(4)**, 245–63.

Hsieh, C. Y., Santoli, R. C., Haslam, S. Z. and Helferich, W. G. (1998). Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. *Cancer Res* **58**, 3833–8.

Lauderdale, D., Jacobsen, S. J., Furner, S. E., Levy, P. S., Brody, J. A. and Goldberg, J. (1997). Hip fracture incidence among elderly Asian-American populations. *Am J Epidemiol* **146**, 502–9.

Marini, H., Minutoli, L., Polito, F. et al. (2007). Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women, *Ann. Intern Med*. **146**, 839–47.

Mei, J., Yeung, S. S. C., Kung, A. W. C. (2001). High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. *J Clin. Endocrinol. Metab.* **86**, 5217–21.

Nelson, H. D., Humphrey, L. L., Nygren, P., Teutsch, S. M. and Allan, J. D. (2002). Postmenopausal hormone replacement therapy: scientific review, *JAMA*. **288(7)**, 872–81.

Ross, P. D., Norimatsu, H., Davis, J. W., Yano, K., Wasnich, R. D., Fujiwara. S. *et al.* (1991). A comparison of hip fracture incidence among native Japanese, Japanese Americans, and American Caucasians. *Am J Epidemiol* **133**, 801–9.

Weaver, C. M. and Cheong, J. M. K. (2005). Soy isoflavones and bone health: the relationship is still unclear, *J. Nutr*. **135**, 1243–7.

Williamson, G. and Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies, *Am. J. Clin. Nutr.* **81(suppl)**, 243S–55S.