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The osteogenic effect of bioactive flavonoid *p*-hydroxycinnamic acid: development in osteoporosis treatment

M Yamaguchi*

Abstract

Introduction

Bone homeostasis is maintained through a balance between osteoblastic bone formation and osteoclastic bone resorption. Ageing induces bone loss due to decreased osteoblastic bone formation and increased osteoclastic bone resorption. Osteoporosis with its accompanying decrease in bone mass is widely recognised as a major public health problem. Pharmacological and nutritional factors may play a role in prevention and treatment of bone loss with ageing. Flavonoid *p*-hydroxycinnamic acid has been found to be a bioactive compound that stimulates bone mineralisation. Among cinnamic acid, *p*-hydroxycinnamic acid, ferulic acid, caffeic acid or 3, 4-dimethoxycinnamic acid, *p*-hydroxycinnamic acid was uniquely found to have stimulatory effects on bone mineralisation and suppressive effects on bone resorption using femoral bone tissues *in vitro*, thereby increasing bone mass. *p*-Hydroxycinnamic acid stimulates osteoblastogenesis and suppresses osteoclastogenesis in bone marrow culture *in vitro*. Oral administration of *p*-hydroxycinnamic acid was found to have preventive effects on bone loss induced with ovariectomy and diabetic state. In this mini-review, the effect of *p*-hydroxycinnamic acid in the prevention and treatment of osteoporosis is discussed.

Conclusion

p-Hydroxycinnamic acid had potent-stimulatory effects on osteogenesis as compared with that of other phenolic acids, indicating a relationship with chemical structure and osteogenic activity. *p*-Hydroxycinnamic acid may be useful as a pharmacologic tool to treat osteoporosis.

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Introduction

Bone homeostasis is maintained through a delicate balance between osteoblastic bone formation and osteoclastic bone resorption. Numerous pathological processes have the capacity to disrupt this equilibrium leading to conditions where the rate of bone resorption outpaces the rate of bone formation leading to osteoporosis, a devastating bone disease that is widely recognised as a major public health threat[1]. Postmenopausal osteoporosis, a consequence of ovarian hormone deficiency, is the archetypal osteoporotic condition in women after menopause and leads to bone destruction through complex and diverse metabolic and biochemical changes[1]. The most dramatic expression of osteoporosis is represented by fractures of the proximal femur for which the number increases as the population ages[2].

Diets and nutritional factors may have potential effects in delaying degenerative bone disorders such as osteoporosis. There is growing evidence that supplementation of nutritional and functional food factors may have preventive effects on bone loss that is induced in animal models of osteoporosis and in human subjects[4–6]. Chemical compounds in food and plants, which regulate bone homeostasis, have been worthy of notice in maintaining bone health and in prevention and treatment of bone loss with increasing age. It appears increasingly probable that as-yet-unidentified factors found in daily consumption of fruits or vegetables may play a role in building of optimal peak bone mass and in preservation of decreased bone mass with ageing.

Cinnamic acid and its related-compounds are present in many plants and fruits. Among cinnamic acid, *p*-hydroxycinnamic acid (HCA), ferulic acid, caffeic acid or 3, 4-dimethoxycinnamic acid, HCA was found to have unique potent-anabolic effects on bone mineralisation, using femoral bone tissues *in vitro*[7]. HCA is an intermediate-metabolic substance in plants and fruits and is synthesised from tyrosine (Figure 1).

HCA has been shown to have stimulatory effects on osteoblastic bone formation and suppressive effects on osteoclastic bone resorption *in vitro*. In addition, HCA has been found to have preventive and treatment effects on bone loss with pathophysiological states including ovariectomy and diabetes state. The objective of this mini-review is to outline the recent advances that have been made concerning the role of HCA in the regulation of bone homeostasis and the effect of HCA in preventing and treating osteoporosis.

Discussion

Bone homeostasis and osteoporosis

Bone metabolism plays a physiological role in maintaining the skeletal structure and regulating mineral homeostasis. Bone homeostasis is

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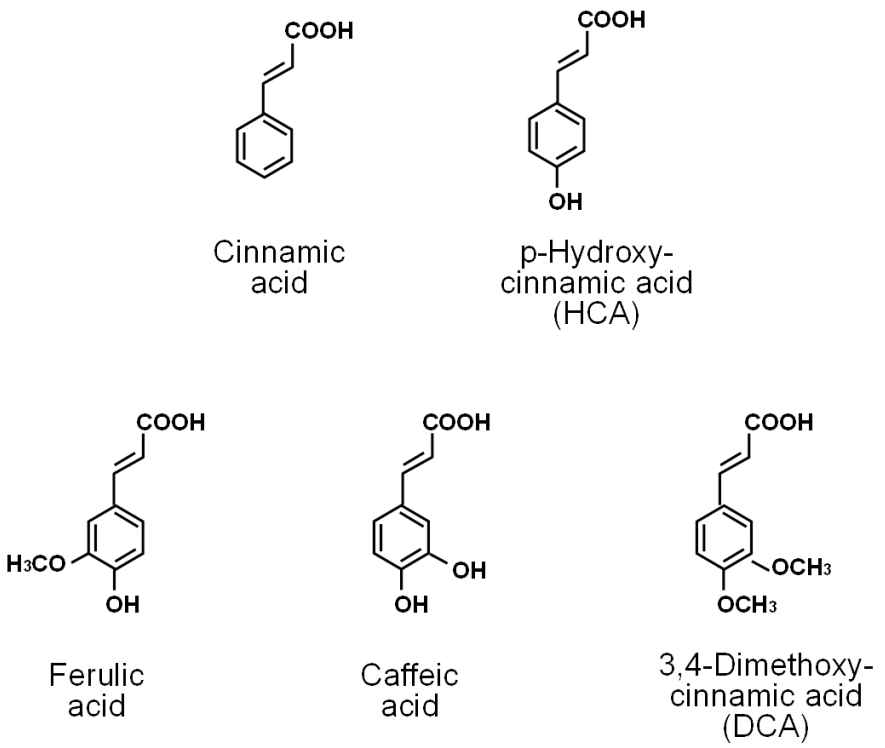

Figure 1: Chemical structure of flavonoid cinnamic acid and its related compounds. The molecular weight of cinnamic acid, *p*-hydroxycinnamic acid (HCA), ferulic acid, caffeic acid, and 3, 4-dimethoxycinnamic acid (DCA) is 148.2, 164.2, 194.2, 180.2, and 208.3, respectively. HCA, which is an intermediate-metabolic substance in plants and fruits, reveals unique osteogenic effects.

regulated by the functions of osteoblasts, osteoclasts and osteocytes, which are major cells in the bone tissues. Bone remodelling and modelling underpin the development and maintenance of the skeletal system[8,9]. Bone modelling is responsible for growth and mechanically induced adaptation of bone and requires the processes of bone formation and bone removal (resorption). Bone remodelling is responsible for removal and repair of damaged bone to maintain integrity of the adult skeleton and mineral homeostasis[9]. This tightly coordinated event requires the synchronised activities of multiple cellular participants to ensure that bone resorption and formation occur sequentially at the same anatomical location to preserve bone mass[9]. In addition, several immune cells have also been implicated in bone disease[9].

Osteoclasts, which develop from haematopoietic progenitors, are uniquely adapted to remove mineralised bone matrix[8,9]. A resorptive stimulus first triggers the recruitment of osteoclasts to a site on the bone surface. This is followed by active resorption by osteoclasts, after which cells withdraw from the bone surface and mononuclear phagocytic cells appear on the newly resorbed surface. Osteoblasts arising from local mesenchymal stem cells assemble at the bottom of the cavity, and bone formation begins. After the resorbed lacunar pit is filled with new osteoid, osteoblasts become flatter and less active with the final newly remodelled bone surface lined by flat lining cells. As bone formation progresses, some osteoblasts are entombed within the matrix as osteocytes; however, the majority dies by apoptosis. This event appears to act as a beacon for osteoclast recruitment and generation of a new basic multicellular unit.

Bone is a major storage site for growth factors[10–12]. Growth factors, which are produced by osteoblasts, diffuse into newly deposited osteoid and are stored in the bone matrix including insulin-like growth factors (IGF-I and IGF-II), transforming growth factor-β1 (TGF-β1), platelet-derived growth factor and bone morphologic protein (BMP). These bone-derived factors, which can be liberated during subsequent periods of bone resorption, act in an autocrine, paracrine or delayed paracrine fashion in the local microenvironment of the bone surface.

The process of bone remodelling that makes bone unique among organs and tissues, and adds so many levels of complexity with respect to interactions along remodelling sequence by systemic influences (hormones), stress action (physical activity/weight bearing), growth factors and cytokines produced by the bone cells or factors that come from nearby cells in the marrow tissues. Regulatory mechanisms of bone homeostasis are very complex.

Bone mass with increasing age is reduced by decrease in osteoblastic bone formation and increase in osteoclastic bone resorption, thereby inducing osteoporosis. Osteoporosis, characterised by reduced bone strength and an increased risk for low-trauma fractures, increases dramatically with age. Osteoporosis is a major cause of increased morbidity and mortality affecting the aging population. The most dramatic expression of osteoporosis is represented by fractures of the proximal femur for which the number increases as the population ages. Bone mass is dramatically reduced after menopause, which depresses the secretion of ovarian hormone (oestrogen) in women. Deficiency of oestrogen

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advances osteoclastic bone resorption. This is very important as a primary osteoporosis. Postmenopausal osteoporosis, a consequence of ovarian hormone deficiency, is the archetypal osteoporotic condition in women after menopause and leads to bone destruction through complex and diverse metabolic and biochemical changes. In addition, osteoporosis has been shown to induce after diabetes (type I and II), obesity, inflammatory disease and various pathophysiological states. In recent years, diabetic osteoporosis is noticed. Diabetes is frequent in the elderly, and therefore frequently coexists with osteoporosis. Type 1 diabetes, and more recently type 2 diabetes, has been associated with increased fracture risk.

Functional food factors (biomedical food factors) may regulate bone homeostasis and have beneficial effects in prevention and treatment of osteoporosis, which is induced in various pathophysiological states.

HCA stimulates osteoblastogenesis *in vitro*

The effect of cinnamic acid or its related compounds on bone mineralisation is examined using rat femoral tissues *in vitro*[7]. Among these phenolic acids, HCA has been found to have unique potent-anabolic effects on bone tissues[7]. This was a first-time finding. The effect of cinnamic acid and its related compounds on bone mineralisation has not been determined so far. Then, the rat femoral-diaphyseal or femoral-metaphyseal tissues were cultured for 48 h in a medium containing cinnamic acid, HCA, ferulic acid, caffeic acid or 3, 4-dimethoxycinnamic acid *in vitro*[7]. Culturing with HCA (10^{-5} or 10^{-4} M) caused an increase in calcium content in the diaphyseal and metaphyseal tissues[7]. Such effect was not observed after culturing with cinnamic acid or other compounds at the concentration of 10^{-5} or 10^{-4} M. Alkaline phosphatase activity and DNA content in the diaphyseal or metaphyseal tissues was also increased after culturing with HCA (10^{-5} or 10^{-4} M)[7]. The effects of HCA (10^{-4} M) in increasing alkaline phosphatase activity, DNA and calcium contents in the diaphyseal or metaphyseal tissues were completely depressed in presence of cycloheximide (10^{-6} M), an inhibitor of protein synthesis. Thus, HCA has been found to have a unique-anabolic effect on bone metabolism, which results from newly synthesised protein components[7]. DNA content in the bone tissues may be partly involved in the number of bone cells including osteoblasts, osteocytes and osteoclasts. HCA increased the DNA content in the femoral-diaphyseal and femoral-metaphyseal tissues of rats *in vitro* and stimulated bone mineralisation. HCA may stimulate proliferation of osteoblastic cells in the bone tissues *in vitro* and has a stimulatory effect on bone formation.

HCA is hydroxylated at position 4 of cinnamic acid (Figure 1). Such chemical form may have an anabolic effect on bone metabolism, suggesting a relationship of structure and activity of cinnamic acid.

HCA has been found to stimulate osteoblastogenesis and mineralisation. Culturing with HCA stimulated osteoblastogenesis in mouse bone marrow culture *in vitro*, suggesting that the compound stimulates differentiation to preosteoblasts of bone marrow mesenchymal stem cells (unpublished report).

Preosteoblastic MC3T3-E1 cells *in vitro* were cultured for 72 h in a minimum essential medium containing 10% foetal bovine serum (FBS) and the subconfluent cells were changed to a medium containing either vehicle or HCA (10^{-7} to 10^{-5} M) without FBS[13]. Culturing with HCA (10^{-7} to 10^{-5} M) did not have a significant effect on cell proliferation of osteoblastic cells in reaching subconfluent monolayers[13]. Also, the number of osteoblastic cells after reaching subconfluent was not changed after culturing with HCA, indicating that the compound does not induce cell death[13]. However, culturing with HCA caused an increase in DNA content in osteoblastic cells[13]. The effect of HCA on increasing DNA content in osteoblastic cells was also seen in the absence of FBS[13]. HCA may have a stimulating effect on DNA synthesis in osteoblastic cells.

Alkaline phosphatase is involved in mineralisation in osteoblastic cells. Alkaline phosphatase activity in osteoblastic cells was significantly increased after culture with HCA (10^{-7} to 10^{-5} M) for 24–72 h[13], indicating that HCA stimulates cell differentiation in osteoblastic cells. Results of alizarin red stain showed that prolonged culture with HCA markedly stimulates mineralisation in osteoblastic cells; the mineralisation was stimulated after culturing with HCA (10^{-8} to 10^{-5} M) for 7, 14, or 21 days[13]. Moreover, culture with HCA has been shown to stimulate osteoblastogenesis and mineralisation in mouse bone marrow culture *in vitro*. Findings support the view that culturing with HCA stimulates the differentiation of osteoblastic cells and that promotes mineralisation in the cells. In addition, HCA caused a significant increase in calcium content in rat femoral tissue culture *in vitro*. Thus, HCA has stimulatory effects on osteoblastic differentiation and mineralisation[13].

HCA suppresses osteoclastogenesis *in vitro*

Osteoclasts are generated from bone marrow stem cells. Receptor activator of the nuclear factor kappa B (NF-κB) (RANK) ligand (RANKL) plays a pivotal role in osteoclastogenesis from bone marrow cells[14,15]. RANKL is produced from osteoblasts in response to osteoporotic factors, such as parathyroid hormone (PTH), prostaglandin E_2 (PGE_2) and 1, 25-dihydroxyvitamin D_3 (VD_3)[14–17]. RANKL acts as osteoclast progenitor and

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stimulates osteoclast differentiation[14,15]. Osteoclastic cells are differentiated from bone marrow stromal cells. Osteoclastogenesis is stimulated through the macrophage colony-stimulating factor (M-CSF) and RANKL *in vitro*[14,15]. A soluble fragment containing part of the extra-cellular domain of RANKL (the carboxyterminal half of the protein, amino acids 158–316) is capable of promoting osteoclastogenesis in the presence of M-CSF[14,15]. The receptor protein RANK for RANKL is expressed on the surface of osteoclast progenitors. Interaction of RANKL with its receptor RANK leads to the recruitment of the signalling adaptor molecules, TNF receptor-associated factors, to the receptor complex and activation of NF-κB and c-Jun N-terminal kinase[18]. The protein kinase C family enzyme has a role in the regulation of osteoclast formation and function potentially by participating in the extracellular signal-regulated kinase signalling pathway of M-CSF and RANKL[17–21].

HCA has been found to have a suppressive effect on bone resorption induced by bone-resorbing factors in the bone tissue culture *in vitro*[7]. PTH or VD_3 are known as bone-resorbing factors[14,15]. Culturing with PTH caused a decrease in calcium content and an increase in the activity of tartrate-resistant acid phosphatase (TRACP), which is a marker enzyme in osteoclastic cells, in the diaphyseal or metaphyseal tissues and a corresponding elevation in medium glucose consumption and lactic acid production by the bone tissues[7]. Such alterations were completely depressed after culture with HCA (10^{-5} or 10^{-4} M). Thus, HCA has been shown to have suppressive effects on bone resorption in the bone tissue culture *in vitro*[7].

Culturing with HCA has also been found to have suppressive effects on PTH-, PGE_2-, or tumour necrosis factor-α (TNF-α)-induced osteoclast-like cell formation from mouse bone marrow *in vitro*[22]. HCA did not have an effect on the proliferation of bone marrow cells, suggesting that the compound did not have a toxic effect on the cells[22]. Suppressive effects of HCA on osteoclast-like cell formation were remarkable at the earlier stage of the differentiation to osteoclasts in bone marrow cultures[22]. In addition, suppressive effects of HCA were also observed at the later stage of osteoclastogenesis[22]. HCA may have an effect on the process of differentiation from mononuclear osteoclast to osteoclast.

Moreover, HCA suppresses osteoclast-like cell formation induced by PTH or PGE_2[22]. Such effect of HCA was partly involved in RANKL expression and/or RANKL action, which is related to the effect of PTH and PGE_2. Culturing with HCA suppressed RANKL plus M-CSF-induced osteoclast-like cell formation in mouse bone marrow culture *in vitro*[22]. Presumably, the suppressive effect of HCA is involved in RANKL expression and/or RANKL action, which are related to the effect of PTH or PGE_2.

TNF-α is an autocrine factor in osteoclasts, promoting their differentiation and mediating RANKL-induced osteoclastogenesis[18]. TNF-α has also been shown to mediate via its p55 receptor in lipopolysaccharide-stimulated osteoclastogenesis[18]. TNF-α-induced osteoclast-like cell formation in mouse bone marrow cultures was significantly suppressed after culturing with HCA[22]. HCA may suppress osteoclastogenesis that is mediated through RANKL and TNF-α.

Phorbol 12-myristate 13-acetate (PMA) is an activator of protein kinase C. PMA stimulated the osteoclast-like cell formation in mouse bone marrow cultures, and PMA-induced osteoclastogenesis was suppressed after culturing with HCA[22]. Moreover, HCA was found to have a suppressive effect on dibutyryl cyclic 3′,5′-adenosine monophosphate-induced osteoclast-like cell formation in mouse bone marrow cultures[22]. Activation of protein kinase C or protein kinase A may be related to RANKL signalling in osteoclastogenesis. HCA may have a suppressive effect on osteoclastogenesis that is mediated through the signalling mechanism of protein kinase C or protein kinase A. Culturing with HCA caused an inhibition of RANKL plus M-CSF-induced osteoclast-like cell formation in mouse bone marrow culture *in vitro*[22]. Such observation may support the view that HCA represses the binding of RANKL to receptor RANK and that suppresses RANKL signalling in osteoclastogenesis.

The effect of HCA in inhibiting RANKL plus M-CSF-induced osteoclastogenesis was completely depressed in the presence of cycloheximide, an inhibitor of protein synthesis in translational processes[22]. Stimulatory effect of RANKL plus M-CSF on osteoclastogenesis was not suppressed in the presence of cycloheximide or 5,6-dichloro-1-β-d-ibofuranosylbenzimidazole, an inhibitor of transcriptional processes[22]. It is possible that suppressive effects of HCA on osteoclastogenesis are partly involved in newly synthesized protein components, which induce suppressor proteins. Whether HCA has an effect on the expression of osteoprotegerin, a regulated suppressor of osteoclast differentiation[14,15] in osteoclasts, remains to be determined.

HCA stimulates osteoblastogenesis and suppresses osteoclastogenesis through suppression of NF-κB activation

The NF-κB signal transduction pathway has long been recognized to be critical for osteoclast development and function[23,24] and double knockout of p50 and p52 NF-κB subunits leads to osteopetrosis due to a severe defect in osteoclast differentiation in these mice[23,24]. Furthermore, it has been reported

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that NF-κB signalling represses basal osteoblast differentiation and mineralisation in MC3T3 cells and antagonises TGF-β1- and BMP-2-mediated MC3T3 mineralisation by down-regulating Smad activation[24]. Other studies have found that NF-κB signalling antagonises Smad activation in Saos2 osteosarcoma cells by a mechanism involving induction of inhibitory Smad7[25].

TNF-α is an inflammatory cytokine that antagonises bone formation *in vivo* and osteoblastic differentiation *in vitro*[24]. These effects are mediated in mainly through NF-κB signalling. Inflammatory levels of TNF-α are known to impact bone formation; however, it has been recently reported that basal TNF-α levels *in vivo* also dramatically lower the basal bone formation rate[24]. HCA may promote the accumulation of basal bone mass, and forestall bone loss during osteoporotic states, in part by antagonising TNF-α-induced NF-κB. Interestingly, HCA prevented the TNF-α-induced suppression of mineralisation in osteoblastic 3T3-E1 cells and repressed TNF-α-induced NF-κB activation in the osteoblastic cells[26,27]. Culturing with HCA was also found to suppress the differentiation of preosteoclasts (RAW267.4 cells) to osteoclasts induced by RANKL through suppression of NF-κB activation[27].

The five members of the mammalian NF-κB family RelA/p65, RelB, c-Rel, NF-κB1/p50 and NF-κB2/p52 are activated through one of the two specific pathways—the canonical NF-κB pathway or an alternative pathway[28]. The canonical pathway activation of the inhibitor of IκB kinase (IKK) complex leads to phosphorylation of NF-κB-associated IκBα, catalysing its ubiquitination and proteasomal degradation, and in the process releasing active NF-κB dimers that translocate to the nucleus and enhance transcription of target genes[28]. In an alternative NF-κB pathway, NF-κB-inducing kinase and IKKα target p100 for proteolytic processing, thereby releasing active RelB-containing dimers[28]. Differential regulation of these two pathways by HCA could provide a more detailed explanation for the suppressive effect observed in TNF-α-induced NF-κB activation.

The cellular mechanism by which HCA stimulates osteoblastic bone formation and suppresses osteoclastic bone resorption is mediated through suppression of NF-κB activation as shown in Figure 2.

HCA prevents osteoporosis *in vivo*

Anabolic effects of HCA on the bone of rats *in vivo* have been shown[29–31]. Rats were orally administered HCA (10, 20 or 50 mg/kg body weight) once daily for 7 days[29]. Administration of HCA did not cause a significant change in body weight or serum calcium and inorganic phosphorus levels. Alkaline phosphatase activity, DNA and calcium contents in the diaphyseal and metaphyseal tissues were increased after administration of HCA (20 or 50 mg/kg)[29]. Diaphyseal calcium and metaphyseal DNA contents were increased with the dose of HCA 10 mg/kg. These findings suggest that the oral intake of HCA induces anabolic effects on bone mineralisation in normal growing rats.

The activity of TRACP, which is a marker enzyme of osteoclastic bone resorption, is enhanced by bone-resorbing factors[13,32]. Oral administration of HCA (10, 20 or 50 mg/kg) caused a significant decrease in TRACP activity in the femoral-diaphyseal and femoral-metaphyseal tissues of rats[29]. This suggests that the administration of HCA induces a decrease in bone-resorbing activity in the femoral tissues of rats *in vivo*.

Mixture of phenolic acids found in the serum of young rats fed blueberries has been reported to stimulate

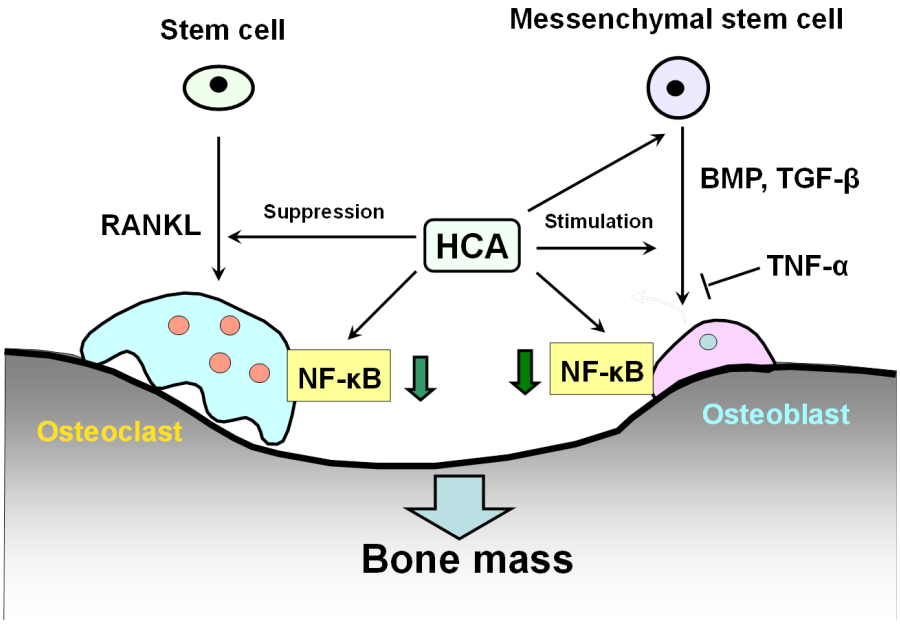

Figure 2: Bioactive flavonoid *p*-hydroxycinnamic acid (HCA) has osteogenic effects. HCA stimulates bone formation and mineralization and suppresses bone resorption in femoral tissues, thereby increasing bone mass. HCA stimulates osteoblastogenesis, which may stimulate differentiation of bone marrow mesenchymal stem cells, and suppresses osteoclastogenesis from stem cells. Osteogenic effect of HCA is mediated through suppression of NF-κB activation. HCA may be useful in the treatment of osteoporosis.

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osteoblast differentiation, resulting in significantly increased bone mass[33]. Greater bone formation in blueberries diet-fed animals is associated with increase in osteoblast progenitors and osteoblast differentiation and reduced osteoclastogenesis[33]. Most of the phenolic acids in the circulation of blueberries diet-fed animals are either metabolites or breakdown products of polyphenols and phenolic acids found in blueberries[33]. Interestingly, HCA was found in the serum after ingestion of diet of blueberries[33].

HCA prevents ovariectomy-induced bone loss.

Preventive effects of HCA on osteoporosis are examined using ovariectomised (OVX) rats, an animal model for osteoporosis[30]. HCA (0.25 or 0.5 mg/kg body weight) was orally administered once daily for 30 days to OVX rats. Analysis using a peripheral quantitative computed tomography (pQCT) showed that OVX caused bone loss in the femoral-metaphyseal tissues[30]. This bone loss was restored after the administration of HCA (2.5 or 5 mg/kg body weight) to OVX rats[30]. Mineral content, mineral density and polar strength strain index in the femoral-metaphyseal tissues were decreased in OVX rats[30]. These decreases were restored after administration of HCA (5 mg/kg) to OVX rats[30]. Especially, the polar stress–strain index is an indicator of bone strength. Moreover, OVX caused a decrease in calcium content or alkaline phosphatase activity in the femoral-diaphyseal and femoral-metaphyseal tissues. These decreases were also restored after the administration of HCA (2.5 or 5 mg/kg) to OVX rats[30]. These observations suggest that the oral administration of HCA causes a functional change in the bone of OVX rats. Thus, HCA has been shown to have preventive effects on OVX-induced bone loss of rats *in vivo*. Presumably, HCA stimulates osteoblastic bone formation and inhibits osteoclastic bone resorption *in vivo*, thereby increasing bone mass in OVX rats.

HCA at a dose of 2.5 or 5 mg/kg body weight had preventive effects on decrease in alkaline phosphatase activity and calcium content or increase in DNA content in the femoral-diaphyseal tissues of OVX rats, although 5 mg/kg of HCA had great potential effects in restoration of OVX-induced bone change[30]. This suggests that intake of HCA of less than 2.5 mg/day/kg body weight has preventive effects on bone loss. Amount of intake of HCA in fruit and vegetables is unknown. However, dietary intake of phytocomponent HCA may have a role in the prevention of bone loss with ageing. HCA may have preventive effects on bone loss with increasing age.

HCA treats diabetic state-induced bone loss in vivo.

Bone loss is induced in the diabetic state[34,35]. Streptozotocin (STZ) induces type I diabetes. Preventive effects of HCA on bone loss induced in STZ-diabetic rats have been examined *in vivo*[31]. Rats received a single subcutaneous administration of STZ (60 mg/kg body weight), and then the animals were orally administered HCA (2.5, 5 or 10 mg/kg body weight) once daily for 14 days. STZ administration caused a decrease in body weight and a significant increase in serum glucose, triglyceride and calcium levels, indicating a diabetic state[31]. These alterations were prevented after the administration of HCA (2.5, 5 or 10 mg/kg)[31]. This was a novel finding. Oral intake of HCA has restorative effects on serum biochemical findings that are involved in diabetes *in vivo*. HCA may have a role in the treatment of diabetic states.

Serum calcium concentration was found to increase in STZ-diabetic rats[31]. Intestinal calcium absorption has been shown to be impaired in the diabetic state[35]. Increase in serum calcium concentration in STZ-diabetic rats may result from release of calcium from the bone tissues; the femoral calcium content was found to markedly decrease in STZ-diabetic rats[35]. Oral administration of HCA to the diabetic rats had a significant preventive effect on hypercalcaemia and bone calcium loss in the diabetic state. Thus, intake of HCA may have restorative effects on bone resorption in diabetic rats.

Calcium content in the femoral-diaphyseal and femoral-metaphyseal tissues was decreased in STZ-diabetic rats[31]. Also, such decrease was prevented after oral administration of HCA (2.5, 5 or 10 mg/kg)[31]. Intake of dietary HCA may have a restorative effect on bone loss in the diabetic state. Alkaline phosphatase activity in the diaphyseal and metaphyseal tissues was decreased in STZ-diabetic rats[31]. Decrease in diaphyseal alkaline phosphatase activity in STZ-diabetic rats was restored after administration of HCA (5 and 10 mg/kg). This enzyme participates in osteoblastic mineralisation. Femoral alkaline phosphatase activity was decreased in diabetic rats, suggesting that osteoblastic bone mineralisation is impaired in the diabetic state.

In addition, the diaphyseal DNA content was also decreased in STZ-diabetic rats[31]. Administration of HCA (2.5, 5 or 10 mg/kg) caused an increase in DNA content in the diaphyseal and metaphyseal tissues in STZ-diabetic rats[31]. DNA content in the bone tissues is an index of the number of existing bone cells. HCA may stimulate an increase in bone cells including osteoblastic cells in the diaphyseal and metaphyseal tissues of STZ-diabetic rats *in vivo*. This may be partly contributed to increase in bone calcium content in STZ-diabetic rats. Presumably, intake of HCA has a stimulatory effect on osteoblastic bone formation in diabetic state.

Thus, intake of HCA has preventive effects on bone loss in STZ-diabetic rats and has restorative effects on serum biochemical findings in the

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diabetic state, suggesting that HCA has treatment effects on the diabetic state. HCA may have a role in prevention and treatment of osteoporosis in the diabetic state.

As mentioned above, HCA has been demonstrated to have anabolic effects on bone mass due to stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption and has restorative effects on bone loss induced in OVX and diabetic rats. Supplemental intake of dietary HCA may have a role in prevention and treatment of osteoporosis.

Conclusion

Diet and nutritional status are critical factors that influence bone development and aged bone loss. Bioactive phytochemicals as food functional factor may play a role in delay degenerative bone disorders with ageing and in treatment of osteoporosis with various pathophysiological conditions. Flavonoid HCA was found to have osteogenic effects due to stimulating osteoblastic bone formation and suppressing osteoclastic bone resorption *in vitro* and restorative effects on bone loss, which is induced with ovariectomy and diabetes *in vivo*. Molecular mechanisms by which HCA stimulates osteogenesis and suppresses osteoclastogenesis may be mediated through suppression of NF-κB activation. NF-κB is a signalling factor that plays a pivotal role in regulation of bone homeostasis regulated through inflammatory cytokines. Whether HCA has an effect on other transcription and signalling systems, however, remains to be elucidated.

HCA had potent-stimulatory effects on osteogenesis as compared with that of other phenolic acids (including ferulic acid, caffeic acid or 3, 4-dimethoxycinnamic acid), indicating a relationship with chemical structure and osteogenic activity. HCA may be useful as a pharmacologic tool to treat osteoporosis. HCA analogues with more potent effect may be developed. Clinical studies in bone disorder are expected through further experiments.

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Abbreviations list

BMP, bone morphologic protein; FBS, foetal bovine serum; HCA, *p*-Hydroxycinnamic acid; IKK, IκB kinase; M-CSF, macrophage colony-stimulating factor; NF-κB, nuclear factor kappa B; OVX, ovariectomised; PGE_2, prostaglandin E_2; PMA, phorbol 12-myristate 13-acetate; pQCT, peripheral quantitative computed tomography; PTH, parathyroid hormone; RANK, receptor activator of the NF-κB; RANKL, RANK ligand; STZ, streptozotocin; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α; TRACP, tartrate-resistant acid phosphatase; VD_3, 1,25-dihydroxyvitamin D_3.

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