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Review

Beneficial Effects of Marine Bioactive Substances on Bone Health, via Osteoarthritis Inhibition and Osteoblast Differentiation

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Abstract Bone health is maintained by balance between bone resorption and bone formation, and bone homeostasis requires balanced interactions between osteoblasts and osteoclasts. Most of drugs and functional foods for bone health have been developed as bone resorption inhibitors, which maintain bone mass by inhibiting the function of osteoclasts. The recent studies have shown beneficial effects of marine natural products on bone health. Therefore, this review is aimed to study effects of marine-derived natural substances on osteoarthritis inhibition via attenuation of MMPs and osteoblastic differentiation via activation of alkaline phosphatase (ALP), osteoclacin (OC), bone morphogenic protein-2 (BMP-2) as an important factor for bone formation, and mineralization. The present review can provide new insights in the osteoblastic differentiation of marine natural products and possibility for their application in bone health supplement.

Key words : Bone health, marine-derived natural substances, osteoarthritis inhibition, osteoblast differentiation, bone mineralization

Introduction

Osteoblasts play a central role in the production of a characteristic extracellular matrix (ECM) and mineralization of the bone matrix. The mineralized bone matrix gives strength to the skeleton and provides a reservoir of minerals and growth factors. Osteoblasts also participate in regulating the differentiation of osteoclasts, the bone-resorbing cells [1]. Bone homeostasis requires balanced interactions between osteoblasts and osteoclasts as well as balance between cartilage degradation and synthesis. Bone homeostasis is disrupted that can lead to degenerative diseases, such as osteoarthritis and rheumatoid arthritis [2-5].

Common characteristics of arthritic cartilage matrix in the severe stage of osteoarthritis and rheumatoid arthritis are a decreased level of macromolecules and increased degradation products. This is believed to be a result of the proteases present in the tissue, as well as to ambient inflammatory mediators such as the cytokines. While several pro-inflammatory cytokines appear responsible for the inhibition of the synthesis of the major cartilage macromolecules including the proteoglycans, others agents such as growth factors have been postulated to modulate cartilage anabolism [6]. The development of inflammatory rheumatic diseases, including an initiation phase associated with genetic susceptibilities, the activation of synovial cells and the development of the pannus are well characterized. Subsequent cartilage and bone destruction leads to an irreversible pathology [7].

Pro-inflammatory cytokines such as interleukin-1 (IL-1), interlukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) produced by macrophages, monocytes and dendritic cells in an arthritic joint are thought to play a significant role in joint diseases. These cytokines stimulate the proliferation of synoviocytes and the secretion of cartilage enzymes. In arthritis, these cytokines induce the principal mediators of cartilage degradation, such as nitric oxide (NO) and matrix metal-

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loproteinases (MMPs), and also inhibit the concentration of tissue inhibitor of metalloproteinases (TIMPs) in arthritic joints [8]. These cytokines also know as osteoclast activating factors that regulate bone resorption. There excessive increase of these cytokines can lead to loss of bone homeostasis and inflammatory disease such as osteoarthritis and rheumatoid arthritis. Thus, investigation of marine bioactive substance effect on osteoblast differentiation that can lead to regain bone homeostasis and possibility for its application in bone health supplement has been attention.

Early histological, cell biological and biochemical studies, performed mainly using cells in culture, indicated that the osteoblast differentiation pathway involved several transitional stages. These studies indicated that type I collagen and alkaline phosphatase are molecular markers of early-stage differentiation, whereas late-stage differentiation is marked by osteocalcin expression and mineralization of the ECM [2, 4, 5, 8, 9].

Natural bioactive substances have come from various resources including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates. Marine organisms have a shorter history of utilization in the treatment and/or prevention of human disease compared to the long standing history medical uses of terrestrial plants [10]. Recently, many scientists make efforts for discovering thousands of bioactive substances from new species, especially marine organism. In this review, we summarized bioactive substances from marine organism have effects on osteoblast differentiation and bone health in human.

Phlorotannins

Phlorotannins are oligomeric compounds with phloroglucinol (1,3,5-trihydroxybenzene) as a basic unit. Several phlorotannins have been identified as the bioactive components in *Ecklonia* species such as *E. cava*, *E. kurome* and *E. stolonifera* [11].

As shown in Fig. 1, Two phlorotannin derivatives, dieckol (**1**) and 1-(3,5-dihydroxyphenoxy)-7 (2,4,6-trihydroxyphenoxy) 2,4,9-trihydroxydibenzo-1,4,-dioxin (**2**) were isolated and characterized from brown alga *Ecklonia cava* that could promote cell differentiation, attenuate MMP-1, MMP-3, MMP-13 expressions, and inflammatory response via MAPK pathway in chronic articular diseases. Phlorotannins reduce the expression of MMPs and inflammation at both the mRNA and protein levels. The inhibitory effects of phlorotannins (**1** and **2** in Fig. 1) on PMA-induced MMPs expression and production of inflammatory mediators in human osteosarcoma, exerts via the downstream regulation by JNK and p38 MAPKs which is serine-threonune kinases of MAPK family, and depends on number and arrangement of OH groups in the phlorotannins [8].

Fucodiphlorethol G (**1,** Fig. 2), a new compound have isolated from the methanol extract of *Ecklonia cava*, a brown alga, collected offshore in Jeju Island. New phlorotannin **1** is named as fucodiphlorethol G has

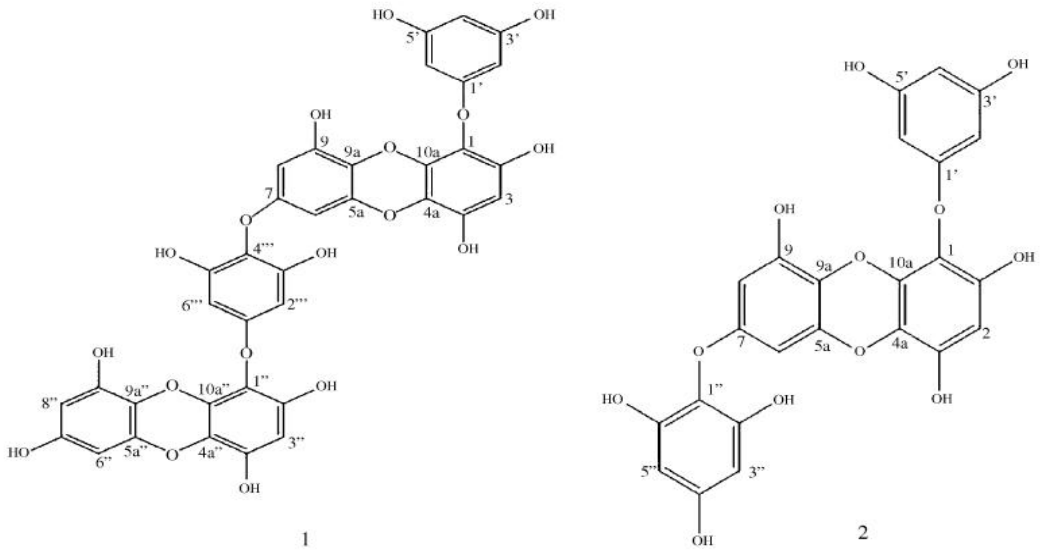

Fig. 1. Structures of phlorotannins from *Ecklonia cava.* (1: dieckol; 2: 1-(3',5'-dihydroxyphenoxy)-7-(2",4",6" trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin)

strong radical scavenging effect (IC_{50} 0.60 μ M) on the DPPH test. The molecular formular of **1** was determined as $C_{24}H_{18}O_{12}$ (un-saturation number 16) on the HR-FAB-MS data [m/z 499.0860 (M+H)+, calcd for $C_{24}H_{19}O_{12}$ 499.0877, Δ −1.7 mmu].

By the examination of 1H and 13C NMR data, it was found that structure of compound **1** is similar to that of triphlorethol-A (**3,** Fig. 2), a trimeric phlorotannin previously isolated. The compound **2** in Fig. 2 was prepared by the treatment of acetic anhydride and pyridine to confirm the connection of C-D ring by HMBC long range correlation using NMR [11].

The nomenclature system for the marine phlorotannins was originally introduced by Glombitza. According to this system, tetrameric phlorotannins (C_{24}) composed of difucol (C_{12}, C_6-C_6 form) and diphlorethol (C_{12}, C_6-O-C_6 form) are named as fucodiphlorethol. In the literature, six different types of fucodiphlorethols, named fucodiphlorethol A-F, have been isolated and characterized in the form of acetylated derivatives. In the case of phlorotannin **1** in Fig. 3, we can describe that O-2 in difucol (C_{12}) is coupled to C-8 in diphlorethol (C_{12}). It is clear that this connection pattern is different from the previous known fucodiphlorethols A-F. Thus, new phlorotannin **1** (Fig. 2) is named as fucodiphlorethol G.

However, the mechanisms of phlorotannin in anti-oxidative and anti-tyrosinase have not investigation yet. And we do not know whether they have beneficial effects on osteoblast differentiation and can useful for cure bone disease or not.

Alkaloids

Norzoanthamine (**1**) as shown in Fig. 4 is one of the zoanthamine class of marine alkaloids isolated from a colonial zoanthid, Zoanthus sp. inhibited the production of interleukin 6 (IL-6) with an IC_{50} value of 13 ug/ml, and its methylester strongly inhibited thrombin-, collagen-, and arachidonic acid-induced human platelet aggregation. Furthermore, norzoanthamine hydrochloride significantly suppressed the decrease in femoral weight and bone biomechanical parameters caused by ovariectomy, without causing an increase in uterine weight. Therefore, norzoanthamine is considered to be one of the most promising drug candidates for osteoporosis [3].

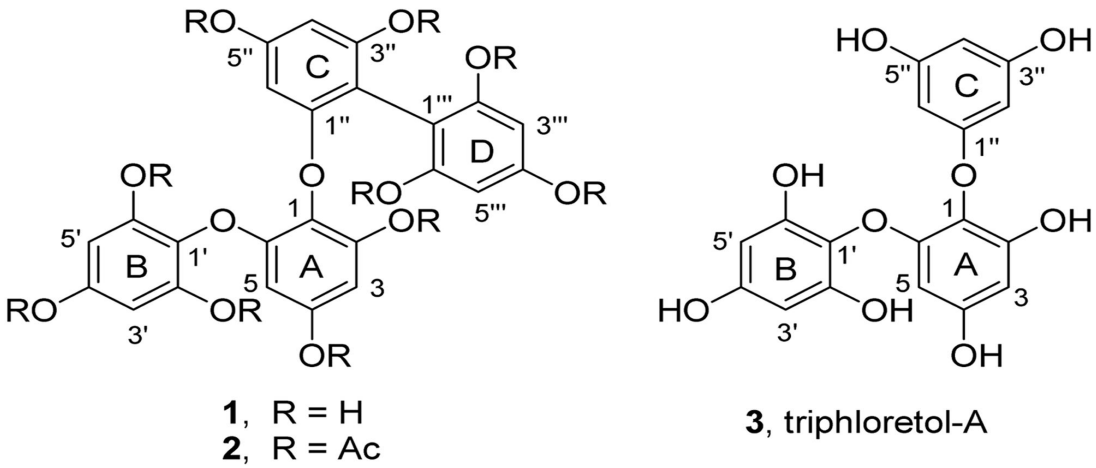

Fig. 2. Structure of phlorotannin from *Ecklonia cava* (1: fucodiphlorethol G; 3: triphlorethol-A)

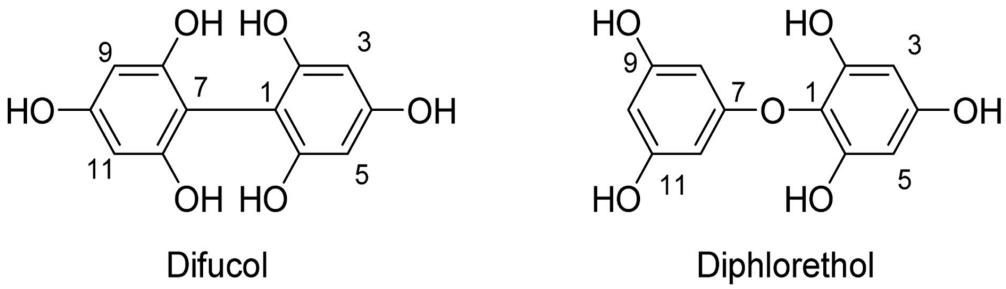

Fig. 3. Analysis of fucodiphlorethols A-F based on their connection pattern between difucol and diphlorethol.

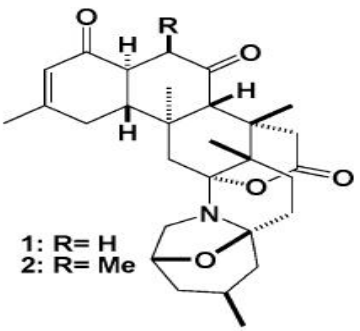

Fig. 4. Structure of norzoanthamine (1) and zoanthamine (2)

Cembranoid diterpenes

Four new cembranoid diterpenes (Fig. 5), namely (-)-7b -hydroxy-8α methoxydeepoxysarcophytoxide (1), (+)-7β, 8β dihydroxydeepoxysarcophytoxide (2), (-)-17-hydroxysarcophytonin A (3) and sarcophytol V (4), along with two known compounds, (+)-sarcophine (5) and sarcophytoxide (6) have isolated from coral *Sarcophyton mililatensis*.

Investigate effects of these compounds on the differentiation-inducing activities of compounds **1-6** on MC3T3-E1 cells by assessing for intracellular ALP activity, collagen content, and calcium deposition. The results determined that compound **1** can increaseALP activity, collagen synthesis and calcium deposition in osteoblastic MC3T3-E1 cells *in vitro*. These studies suggest that compound **1** may be able to stimulate osteoblastic bone formation and play an important role in bone remodeling [12].

Polysaccharides

Fucoidan, a sulphated polysaccharide, contains large proportions of L-fucose and sulphate, together with minor amounts of other sugars like xylose, galactose, mannose and glucuronic acid that extracted from the marine brown alga Undaria pinnatifida. Fucoidan has showed significantly induced osteoblastic cell differentiation and possibility for its application in bone health supplement. A non-toxic sulphated polysaccharide, fucoidan, can increase activity of alkaline phosphatase and level of osteocalcin. It is also showed positive effects on bone morphogenic protein-2 as an important factor for bone formation, remodeling and mineralization [2].

Glucosamine sulphate (SGlc) has been known to be effective in controlling osteoarthritis symptoms in several clinical (Fig. 6). SGlc derived from chitin, was extracted from marine invertebrate animals, can promote osteoblast differentiation via anti-inflammatory effect. SGlc can increase ALP activity, collagen synthesis, osteocalcin secretion, and mineralization in osteoblastic cells in vitro. Furthermore, it was observed that SGlc exhibited anti-inflammatory effect on production of TNF-α, IL-1β, and PGE_2 in macrophage, RAW264,7

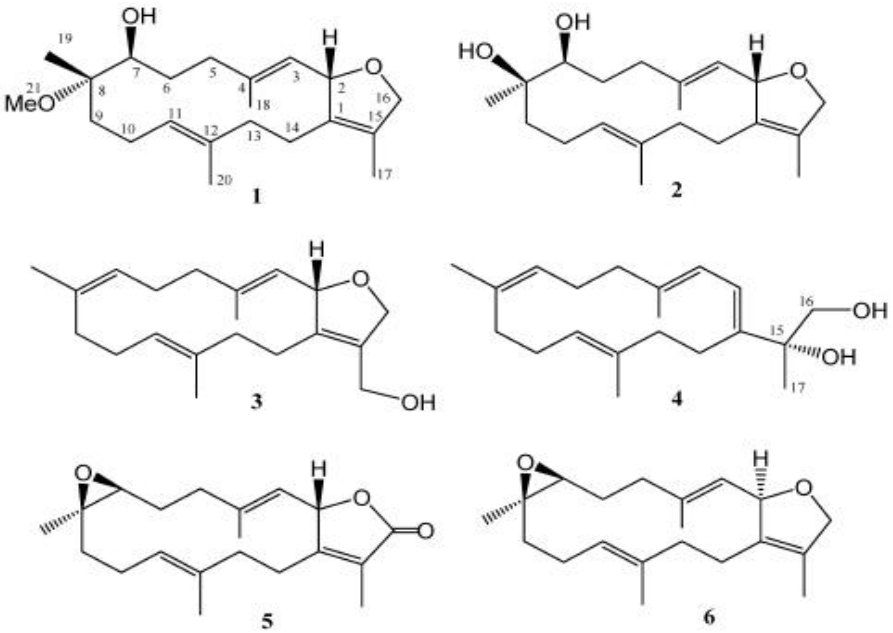

Fig. 5. Structures of Compounds 1-6

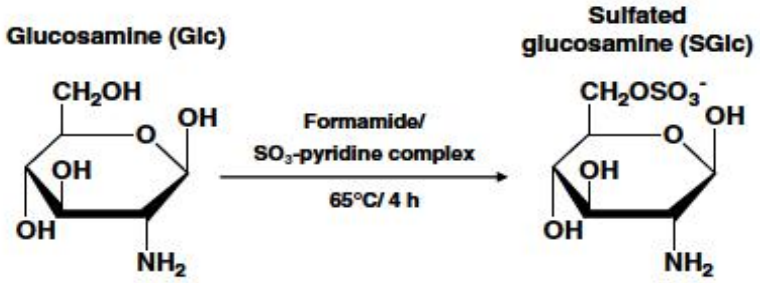

Fig. 6. Synthesis of glucosamine sulphate from glucosamine hydrochloride

cells [9].

Peptides

SHP-1 (LEDPFDKDDWDNWKS), a novel peptide derived from seahorse hydrolysate was able to suppress not only the expression of collagenases 1 and 3, but also the production of NO via down-regulation of iNOS. This peptide has inhibitory effects on collagen release in arthritis that is associated with restraining the phosphorylation of NF-kB and p38 kinase cascade [5].

A bioactive peptide LEDPFDKDDWDNWK derived from seahorse hydrolysate induces differentiation and inhibited NO production in osteoblastic MG-63 and chondrocytic SW-1353 cells. The purified peptide effectively promotes differentiation, suppresses MMPs (-1, -3 and -13) and inhibits TPA-induced production of inflammatory mediators (iNOS, COX-2) through blockade of NF-_B and the MAPK. Therefore, this isolated peptide could be a therapeutically effective agent for modulation of differentiation and inflammation activity in arthritis [4].

Discussion

Marine ecosystems provide a wide range of goods and services which are essential for the human population. These include seafood, fuel, biological products for medicinal purposes, nutrient and waste management, climate regulation, development of tourism activities and nonmaterial benefits such psychological and emotional benefits [13]. The oceans are a unique resource that provide diverse array of natural products, primarily from invertebrates such as sponges, tunicates, bryozoans, mollusca, and from marine bacteria and cyanobacteria [14]. The Marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit unique structural features that are not found in terrestrial natural products. Each year, an increasing number of novel marine metabolites and marine natural compounds are reported in the literature indicating that the marine environment will continue to be a prolific source of new natural products for many years to come. Many additional published papers describe the synthesis or the biochemical and pharmacological characterization of these new substances. Several marine natural products are entering clinical trials, others are lead compounds for medical chemistry efforts, and many others are becoming important tool compounds for the study of biochemical and cellular processes [15].

There has been an increasing recognition of the inter-relationship between human health and the oceans. Understanding the oceans roles in human health first laid out specific focus areas of scientific interest and data gaps. Finally, there has been the realization that, similar to, or perhaps even more than the endangered tropical rainforests, the earth's oceans are a source of great biological diversity with an almost unexplored potential to provide significant therapeutic, as well as nutritional, benefits for humans and other animals [16]. The study of marine organisms, especially their defense mechanisms, provides opportunities for discovering and developing new pharmacological tools, as well as for finding new molecules that potentially could become drugs.

Although marine bioactive substances have been exploited for a variety of purposes including use as food, insecticides and medicines, the investigations for application in bone health have just attended recently [15]. There are still more potential of marine bioactive substances have yet to be identified. It is also has limit in knowledge about mechanisms or pathways or factors that regulate osteoblast differentiation and bone disease.

There are many factors that have effects on osteoblast differentiation in many different signals but we have not known clearly about them and their relationship. This researching fields still have mysteries we have not be able to unravel. These are also interesting fields that many scientists can interst and explore to understand exactly in future.

Several signaling molecules play major roles in controlling skeletal development such as bone morphogenetic protein (BMP) family, other members of the transforming growth factor β (TGF-β) superfamily, fibroblast growth factors (FGFs), etc. In addition, components of the ECM play crucial roles in either modulating or maintaining the phenotype and correct organization of cells in cartilage and bone [1].

Normal articular cartilage consists of extracellular matrix composed of molecules including collagens, proteoglycans, link protein and hyaluronic acid, which are synthesized by the chondrocytes. Cartilage integrity is maintained by the strict regulation of the balance between degradation and synthesis of these components [6]. Progressive degradation of the ECM that comprises joint tissues, including articular cartilage, bone and even intraarticular ligaments and tendons, is a major feature of the arthritic diseases, leading to permanent loss of function. Although proteinases of all mechanistic classes play a role in the degradation of connective tissue macromolecules, it has long been thought that the major activities involved in this process belong to the family of MMPs. These enzymes are secreted by both the resident cells of joint tissues as well as by invading cells, they are active around neutral values of pH, and they have the combined ability to degrade all the components of the ECM. MMPs play significant roles in both developmental and repair processes, and it appears that aberrant regulation, which can occur at many levels, leads to their hyperactivity in diseases such as rheumatoid arthritis (RA) and OA [7, 17].

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