MY MENTOR AND I **Perspective**

Compounds isolated from corals a potential therapeutic strategy in bone diseases

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ABSTRACT

The ocean provides food and shelter to diverse marine species, and it is an exceptional storehouse of potential bioactive natural products that needs to be explored.

Many marine organisms live in extreme conditions, and they have needed to adapt to a complex habitats, as a result, they produce a wide variety of unique active molecules that are used as a defense against predators or to capture their prey. Many of these molecules have biological activity, and they have been described as potent anticancer, antimicrobial, anti-inflammatory, antioxidants, and antihypertensive agents. Our goal is to identify some compounds that can inhibit bone resorption as well as compounds that increase bone formation in patients with osteoporosis that have low bone mass and are at risk of fractures. Although many studies aim to characterize natural compounds that can be used for the treatment of cancer, there has been less focus in the area of bone and bone diseases. For this reason we are excited to contribute to the development of this field and hopefully, very soon, to have new potential drugs for the treatment of patients that suffer these diseases.

Introduction

Bone and bone diseases.

Bone is a dynamic tissue, which is constantly regenerated during our life to regulate and maintain its strength and integrity. This process is known as bone remodeling. To be carried out it requires the action of bone cells coordinated by signaling molecules. There are three types of bone cells: the *osteoblasts*, cells responsible for producing and mineralizing the components of the bone matrix, the *osteocytes* that are osteoblasts that kept on differentiating and became trapped within the bone matrix and function as mechanosensors, and the *osteoclasts,* the bone resorbing cells **(Boskey & Robey, 2013; Wan et al., 2016**).

Bone cells coordinate bone remodeling by communicating with one another through either direct cell contact or signaling molecules **(Boskey & Robey, 2013)**. However, these mechanisms can be interrupted by diverse factors, which trigger different bone pathologies, including osteopetrosis, Paget's disease, bone metastases, and osteoporosis **(HHS, 2004).**

Conventional therapy in bone diseases

In the last years, treatments for bone diseases have focused on antiresorptive or anabolic therapies. The most common antiresorptive drugs are bisphosphonates that induce osteoclast apoptosis; and denosumab, an anti-RANKL antibody that inhibits the recruitment and activity of osteoclasts in the resorption area. Analogs of parathyroid hormone and parathyroid hormone-related protein (teriparatide and abaloparatide) are the only anabolic therapies approved (**Harslø & Langdahl, 2016; Cawthray et al., 2017**).

Despite the positive results obtained we must improve these therapies and solve important questions, such as the optimal duration of treatments, the safety of their long-term administration. These therapies can increase bone mass to a certain point and once they are suspended the bone mass decreases again, meaning that the effects are not permanent. For example in the case of denosumab, bone density declines rapidly once the treatment is suspended and bone turnover markers increase above baseline **(Tripto-Shkolnik, et al., 2018)**. Another example of this, is the case of teriparatide, a sustained administration increases bone resorption whereas intermittent dosage rises bone formation, this limits the therapeutic effect, and some patients with reduced bone mass or suboptimal response to teriparatide remain with very low bone mineral density after the treatment **(Harslø & Langdahl, 2016)**.

Currently, the maximum recommended duration for anabolic therapy is two years and for antiresorptive treatments (bisphosphonates) five to ten years. This gives us an example that the treatments are palliative. None is appropriate for all patients, and that they still have no long-lasting effect on the specific molecular mechanisms of bone diseases. Therefore their use presents physicians with a dilemma: when to use them for maximum benefit, whether it is safe to repeat them and for which duration **(Harslø & Langdahl, 2016; Manolagas, 2018)**. So we must continue searching for new therapeutic agents, which requires elucidating the molecular mechanisms of the diseases that affect bones.

Ocean as a source of new therapeutic drugs

The oceans cover more than 70% of the surface of the Earth, providing a vast space for the diverse marine lifeforms **(Fusetani & Kem, 2009)**. Many marine organisms have adapted to complex habitats exposed to extreme conditions (i.e., high temperature or

salinity, absence of light), and as a result, produce a wide variety of secondary metabolites, not present on the landmass **(Senthilkumar & Kim, 2013)**. Others use small peptides or high molecular weight proteins as means of defense against predators, to decrease the excessive growth of other organisms or to capture their prey **(Fusetani & Kem, 2009)**.

With the development of technology, it is possible to extract a large variety of compounds such as: peptides, alkaloids, nucleosides, peroxides, terpenes, fatty acids, sterols, carbohydrates, amino acid derivatives, cyclic peptides, and polyketides from tunicates, marine sponges, seaweeds, nudibranchs, bryozoans, corals, or sea snails, among others **(Kang et al., 2018; Calcabrini et al., 2017)**,. Many of these compounds are associated with anticancer, antimicrobial, anti-inflammatory, antioxidants, antiviral, antituberculosis, and antihypertensive properties **(Jha & Zi-Rong, 2004; Lee et al., 2017)**.

Cytarabine and vidarabine were the first drugs of marine origin to be approved by the Food and Drug Administration (FDA), in 1969 and 1976 respectively, and both compounds were isolated from the Caribbean sponge *Tethya crypta* **(Montaser et al., 2011)**. Cytarabine is a synthetic version of a cytosine arabinoside. It acts by arresting the cell cycle during the S phase and inhibits DNA synthesis by preventing the activity of DNA polymerase **(Lee at al., 2015)**. Cytarabine is used for the treatment of leukemia. In 2017, a combination of daunorubicin and cytarabine, encapsulated in liposomes, was approved for the treatment of some types of acute myelocytic leukemia **(Gomes et al., 2016)**. On the other hand, vidarabine is an antiviral agent,. It is a synthetic version of an adenine arabinoside, and is used in the treatment of herpes virus infection **(Montaser et al., 2011)**.

An extended period of time passed before a new marine compound was approved again for use in patients. In (indicate the year), ziconotide a synthetic version of the ω-conotoxin MVIIA (ω-MVIIA) started being used for the treatment of severe chronic pain. This conotoxin was isolated from the venom of the sea snail *Conus magus* **(McGivern, 2007)**. In 2015, trabectedin was approved by the FDA, for the treatment of liposarcoma and leiomyosarcoma, two subtypes of soft tissue sarcoma. This compound is a partially synthetic analog of an alkaloid isolated from the marine tunicate *Ecteinascidia turbinata* **(Montaser et al., 2011)**.

Currently, new compounds of marine origin are in clinical phase III trials. For example, Eribulin mesylate, an analog of the marine natural product halichondrin B, initially extracted from marine sponges is a microtubule-depolymerizing drug; eribulin treatment resulted in a survival advantage for patients with metastatic or locally advanced breast cancer **(Dybdal-Hargreaves et al., 2015)**. Soblidotin is a synthetic derivative of dolastatin 10, a compound originally isolated from the sea hare *Dolabella auricularia*. It induces the collapse of the vasculature inside the tumors, in addition to its tubulin inhibitory activity (missing a reference). Tetrodotoxin is a potent neurotoxin of marine origin, but at an adequate dose it is a strong analgesic to treat pain in patients with cancer **(Malve, 2016)**. Promisingly, in consequence of the large number of compounds that have been isolated and are under study, it won't be a surprise if we see a rise in approved drugs from marine origins over the next years

The vast amount of compounds isolated from marine organisms shows very particular chemical and biological characteristics. However, because these species, compared to terrestrial species are practically inaccessible, it is probable that many

compounds that could be used to treat diverse human diseases or as a molecular basis for the development of new drugs, are still unknown **(Fusetani & Kem, 2009)**.

Corals as a potential source of bioactive compounds

The Cnidaria phylum includes organisms that are among the most venomous animals, the species of this phylum are divided traditionally into Hydrozoa, Scyphozoa, and Anthozoa. The Anthozoa class includes sea anemones, hard corals, soft corals and sea pens **(Frazão et al., 2012)**. Soft corals have been studied extensively for their secondary metabolites like terpenoids (especially diterpenoids), which exhibit a wide range of biological activities **(Fusetani & Kem, 2009)**.

Some of the metabolites extracted from soft corals have been evaluated as anti-inflammatory compounds, and as anticancer agents on different cell lines derived from melanoma, leukemia, breast, colon or lung cancer, resulting in promising results. Surprisingly, although bones are commonly affected by the metastasis of some of these cancers, none of the isolated metabolites have been evaluated for their efficacy in bone metastasis.

A coral toxin with the potential to induce bone resorption

Palytoxin is an unusual and highly toxic metabolite produced by soft corals of the genus *Palythoa*, and initially isolated from *Palythoa toxica*. The mechanism of action of palytoxin has been widely studied, and its properties are exerted through a variety of process, among them, binding to the Na^+/K^+ ATPase , on the cell membrane, converting it into an ion channel, and increasing the permeability of Na^+ through the cell membrane **(Fusetani & Kem, 2009)**. Besides, palytoxin induces a significant increase in mRNA levels that encode proteins related to inflammation in cells of the immune system, suggesting that this toxin has the potential to cause a proinflammatory activity **(Crinelli et al., 2012)**. When the effect of palytoxin was evaluated on mouse calvarias cultured *ex vivo*, it proved to be a potent inducer of bone resorption at low doses **(Lazzaro et al., 1987)**. Therefore palytoxin could be used for the treatment of diseases where bone formation is increased (Figure 1).

Compounds isolated from corals that can inhibit bone resorption

(-)-7b-hydroxy-8amethoxydeepoxysar-cophytoxide. This metabolite is a cembranoid, a molecule that includes a cembrene ring composed of 14 carbon atoms and that belongs to the terpenoid family. It was isolated from the soft coral *Sarcophyton mililatensis*. The cembranoids are biologically active metabolites that are used by corals as a defense system against predators and have been mainly studied for their antitumor effect. In the search for new agents that induce bone formation, the effect of cembranoid (-)-7b-hydroxy-8amethoxydee-poxysarcophytoxide was evaluated on MC3T3-E1 cells, osteoblast precursors, demonstrating that it can increase ALP activity, collagen synthesis and calcium deposition **(Cuong et al., 2008)**. This study suggests that this metabolite induces the differentiation of osteoblast precursor cells and the activity of osteoblasts, and that this compound could be used to increase bone formation, although more studies are required to confirm it (Table 1).

Norzoanthamine. In 1995, an alkaloid of the zoantamines group named Norzoanthamine was extracted from the soft coral *Zoanthus sp.* **(Fukuzawa et al., 1995)**. Since then, possible applications for this molecule have been sought, and it has been shown to induce the aggregation of human platelets **(Villar et al., 2003)**. Studies of its action

mechanism showed that norzoanthamin protects collagen fibers, elastin, and albumin from cleavage by proteases **(Inoue et al., 2014)**. Besides it aggregates with the collagen, increasing the deposition of hydroxyapatite **(Kinugawa et al., 2009)**. These effects of norzoanthamine could explain why it prevented a decrease in bone mass and bone strength in ovariectomized mice **(Kuramoto et al., 1998)**. The protective activity on collagen fibers of Norzoanthamine and collagen being one of the main components of the extracellular bone matrix make it a molecule with potential application in conditions where bone loss is increased, like osteoporosis.

11-epi-sinulariolide acetate (Ya-s11). Ya-s11 is also a cembranoid, isolated from the soft coral *Sinularia querciformis*. Ya-s11 inhibits significantly the expression of proinflammatory proteins induced by nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in the murine macrophage cell line RAW264.7 stimulated by lipopolysaccharide (LPS), In a model of adjuvant-induced arthritis (AIA) using female Lewis rats, Ya-s11 reduced the extent of the disease **(Lin et al., 2013)**. Ya-s11 decreased the expression of osteoclast-related proteins such as cathepsin K, matrix metalloproteinases-9 (MMP-9), tartrate-resistant acid phosphatase (TRAP), and tumor necrosis factor-a (TNF-a) in ankle tissues of AIA-rats, in a dose-dependent manner, leading to a decrease of bone destruction due to arthritis **(Lin et al., 2013)**. Although the authors suggested that it could be a compound for the treatment of AIA, it could be evaluated in other models of bone diseases where bone destruction is increased.

Lemnalol. Lemnalol is a sesquiterpenoid that is produced by the soft corals *Lemnalia cervicorni* and *Lemnalia tenuis*, which was shown to have anti-inflammatory properties by inhibiting the expression of the proinflammatory proteins iNOS and COX-2 **(Jean et al., 2008)**. Therefore, its effect was evaluated in a model of Gouty arthritis. The

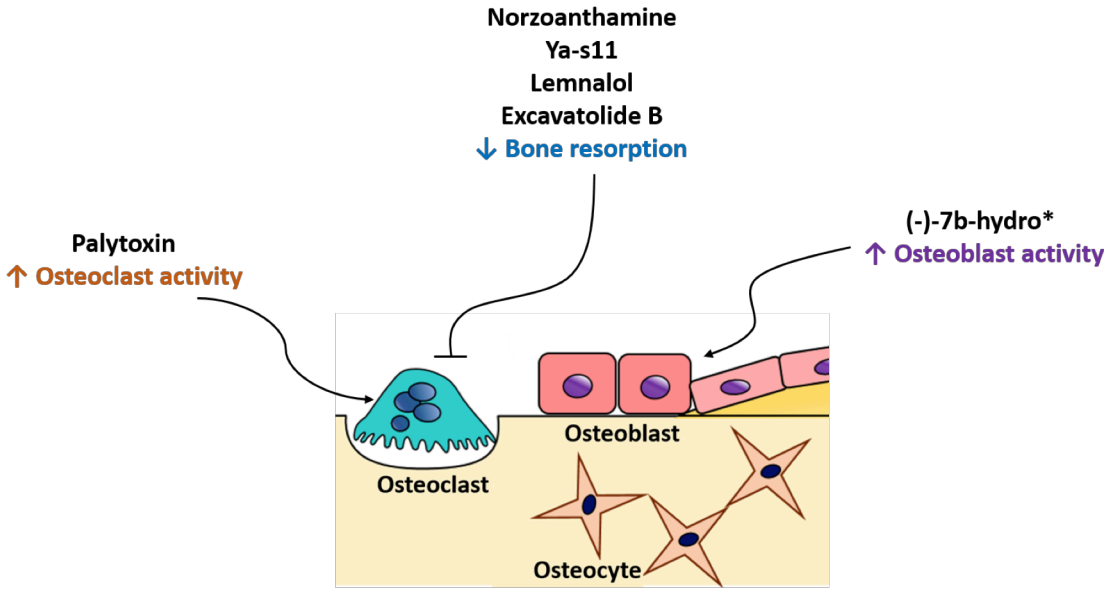

Image 1. Corals and bone remodeling. Corals are marine organisms from which various compounds have isolated that can modulate the bone cells activity and could be potential agents in the treatment of bone diseases. (-)-7b -hydroxy-8a-methoxydeepoxysarcophytoxide*, 11-epi-sinulariolide acetate (Ya-s11).

Gouty arthritis is an inflammatory disease that can cause bone erosion. Lemnalol showed to decrease the expression of the osteoclastic markers TRAP, cathepsin K, and MMP-9, and of TGF-ß1, suggesting a modulation of osteoclastogenesis. Treatment with Lemnalol decreased bone loss derived from Gouty arthritis, and could probably be used in other diseases with increased bone resorption **(Lee et al., 2015)**.

Excavatolide B. The diterpene Excavatolide B was isolated from soft coral *Briareum excavatum*. It is known that, as a bioactive molecule, it can generate anti-inflammatory and analgesic effects **(Lin et al., 2015)**. Therefore, it was evaluated in the context of rheumatoid arthritis, where the chronic inflammation leads to an increase in bone resorption in the joint space, due to the activation of osteoclasts. The study found that Excavatolide B inhibits the differentiation of RAW264.7 into osteoclasts, induced by LPS. Also, it decreases osteoclast differentiation in different models of arthritis induced by adjuvant or by type II collagen, leading to the conclusion that Excavatolide B reduces osteoclastogenesis via the downregulation of the inflammatory factors IL-17A and M-CSF to activating the MAPK and HO-1 / HMGB-1 pathways *in vivo* **(Lin et al., 2017)**.

Although the previously mentioned compounds have been evaluated mainly in inflammatory diseases, they demonstrate a potential in the regulation of bone cells (Figure 1). Therefore they should not be ruled out as agents with therapeutic potential in bone diseases

A little more about corals

In addition of a large variety of metabolites with biological activities and therapeutic potential, corals could also contribute to bone health thanks to their very own "skeleton". A large group of corals that includes the genera *Porites, Goniopora, Acropora* and *Lobophylia* are constituted by an extended network of channels and pores, made of spicules of carbonates of lime or silica. This structure forms an internal skeleton that mimicks the architecture of our spongious bones. For this reasen, it was postulated that coral skeleton could be used as replacement for human bones. Upon implantation, it would be repopulated by blood vessels and bone cells, leading to the formation of new bone **(Green et al., 2017)**. Zheng et col. **(2011)** implanted human bone marrow mesenchymal cells, modified to express basic fibroblast growth factor (FGF) into porous coral scaffold. It was successfully repopulated by bone and endothelial cells and the authors found evidence of deposition of collagen type I and II.

Or its conversion to hydroxyapatite and fusion with nanocoatings of hydroxyapatite to strengthen the bone replacement against compression **(Green et al., 2017)**.

Perspectives

Despite the considerable scientific advances of our era, there continues to be a growing need for new therapies for pathologies that affect bones (osteoporosis, bone metastasis, osteoarthritis, osteosarcoma). Marine organisms such as corals offer a wide range of new unique compounds that could serve as new therapies. That's the reason why, in our laboratory, we are studying the effects on bone regeneration of compounds isolated from corals, sea snails, or algae.

Conflict of Interest

None Reported

Acknowledgements

The authors would like to thank the *Center for Scientific Research and Higher Education of Ensenada* (CICESE) and the *Consejo Nacional de Ciencia y Tecnología* (CONACYT) for their funding support. The authors are

also particularly grateful to Damian Loera (Department of Communication, CICESE) for his invaluable help to realize the associated video.

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