Cannabinoids: new friends in bone fracture and in joint disease prevention?

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ABSTRACT

In recent years the endocannabinoid system (ES) has been recognized to play an important role in the regulation of several physiological processes, including pain perception, appetite control, and motor function development. This system has recently been recognized to be present in bone and joint tissues, playing a role in the regulation of bone and joint physiology. The ES seems to play its role mainly by acting on its receptors and thanks to the demonstrated ability of bone cells to synthesize the principal endocannabinoids (i.e., anandamide and 2-arachidonoylglycerol), which can act on bone remodeling and metabolism. Cannabinoids have also been shown to be produced within synovial tissues, and recent studies have shown that cannabinoid receptor ligands are effective in the treatment of inflammatory arthritis. In recent years synthetic endocannabinoid-like compounds and phytocannabinoids, which are the principal components of Cannabis sativa, have also started to be studied as molecules that could play a role not only in bone physiology, but also in the pathogenesis of osteoporosis, the most common bone disease, and in the inflammatory processes underlying osteoarthritis. Accumulating evidence that cannabinoids and their receptors play an important role in bone metabolism and in the regulation of the immune response is now starting to show us the true future therapeutic potential of cannabinoids, and of the phytocannabinoids contained in Cannabis sativa, in the treatment of bone loss and joint diseases. Here we summarize the role of the cannabinoids and their receptors in bone metabolism, osteoporosis, and joint disease.

KEYWORDS

Cannabinoids, bone, bone remodeling, joint tissues, endocannabinoid system, osteoarthritis, osteoporosis.

Introduction

Cannabis sativa was already widespread in China in 4000 B.C., where it was found in textiles and its seeds were used as medicines to treat rheumatic pains, intestinal constipation, malaria, muscle spasms, asthma, and pain. Whereas *Cannabis sativa* consumption in China was linked to medicine, without particular attention to the psychotropic effects it can induce, in India, it was particularly popular precisely for these effects. In fact, India is where, because of these effects, mainly related to consumption of cannabis leaves, which are much more abundant in Delta-9-tetrahydrocannbinol (D9-THC) than the seeds, *Cannabis sativa* became highly popular as a recreational substance. Indian people in fact described this plant as a "source of happiness, bringer of joy and freedom", and this is why *Cannabis sativa* was listed in sacred texts as a sacred plant, used during ceremonies and rituals. Nevertheless, the seeds were used for medicinal purposes as well (i.e., as an anticonvulsant, antiseptic, diuretic, and to treat pain and muscle spasms, etc.) [1,2]. How did the use of *Cannabis sativa* change over time?

In ancient Rome, between 200 B.C. and 200 A.D., cannabis was used as a natural treatment for earache and pain, in line with what Greek physician Galen also reported in his medical text. The medical use of cannabis spread over the centuries as awareness of its psychotropic effects grew. These effects explain why its administration is highly controlled. Growing interest in the therapeutic potential of cannabis plants led to the first studies on possible future applications of cannabinoids as therapeutic agents [3]. In 1621, Robert Burton in his text "Anatomy of Melancholia" describes cannabis as an important natural medicine against depression [4]. In 1682, the New London Dispensatory defined *Cannabis sativa* as a "valid treatment of cough and jaundice, but which can fill the head with mists", highlighting how prolonged and excessive use of it can have significant psychotropic effects. It was 1764 when the New English Dispensatory described how a paste made from cannabis leaves and applied to damaged skin could treat skin inflammation, tumors, and joint pain. It was the 18th century when medical societies around the world started to seriously consider and describe *Cannabis sativa* as a natural medicine. During the first international meeting on the topic, held in the USA in 1860 and organized by the Ohio Medical Society, *Cannabis sativa*

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was for the first time recognized as a therapeutic plant and as a natural medicine to treat joint pain, asthma, chronic cough, gonorrhea, cholera, neuralgia, childbirth pains, rabies, and convulsions. The late 20th century brought a fundamental discovery, when Mechoulam and Gaoni, in 1964, first identified and described D9-THC as the active chemical component of *Cannabis sativa*. This paved the way for all the subsequent discoveries regarding its other active chemical components, which in turn led researchers to wonder how these molecules work in our body [5]. Then came the first discoveries concerning the endocannabinoid system (ES) and its components [6-8]. Following studies on the ES and on the active chemical components of *Cannabis sativa*, which have been called phytocannabinoids (PCBs) (i.e., cannabidiol (CBD), Δ9-THC, cannabigerol (CBG), cannabidivarin (CBV), cannabichromene (CBC), etc.), to distinguish them from the endocannabinoids (ECBs), which are endogenous, being synthesized inside the body (i.e., arachidonnoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG)) [9,12], researchers started to understand how they had therapeutic potential not only as analgesics and anticonvulsants, but also potentially in the treatment of other diseases. In fact, thanks to studies demonstrating how the ES plays an important role in regulating several physiological processes, such as appetite control, pain perception, and immune regulation, the point was reached, in 1985, where the Food and Drug Administration (FDA) was able to approve the use of Dronabinol, a synthetic stereochemical variant of Δ9-THC, which is not psychoactive, in cancer patients undergoing cycles of chemotherapy. In the 1990s, clinical trials showed that dronabinol was not only an antiemetic drug, but also an effective appetite enhancer in cancer patients affected by cachexia. These effects were also demonstrated in AIDS patients, and so in 1992 the FDA approved the use of Dronabinol in these patients, too [1]. In the same years, Nabilone, another synthetic cannabinoid with the same therapeutic effects, received FDA approval, and was subsequently (in 2007) approved in Italy, too.

In recent years, another two drugs, which are a CBD and tetrahydrocannabinol (THC) mix, have been approved by the FDA to treat, respectively, the epileptic crises and muscle spasms that characterize Dravet syndrome and Lennox-Gastaut syndrome. In the last few decades several research groups have reported the presence of the ES in bone and in synovial joints, too, showing the important role it can play in bone metabolism. Several *in vitro* and *in vivo* studies have started to demonstrate that the ES, PCBs, and ECBs could play an important role in several physiological process underlying the maintenance of skeletal health. In this work we will review the role that cannabinoids play in the regulation of bone metabolism and will discuss their potential therapeutic effects when used to treat bone and joint diseases.

Bone remodeling

The skeleton is a dynamic and metabolically active organ that, to fulfil all its fundamental functions (i.e., movement, support, protection of internal organs, etc.), undergoes continuous change over the course of life. This process, whereby skeletal change protects the structural integrity of the skeletal system, and helps to maintain the body's balance of calcium and phosphorus, is called bone remodeling. Bone remodeling is characterized by five phases: (i.e., activation, resorption, reversal, formation, and quiescence) and it is essentially carried out by two bone cell populations: osteoclasts (OCs) and osteoblasts (OBs). The first process, resorption, starts with the recruitment of OC progenitors from hematopoietic lineage to the bone surface of the remodeling site. Once fusion of these progenitors has occurred, forming activated multinucleated OCs, these OCs attach to the bone surface and start the resorption of old or damaged bone tissue through a combination of lysosomal enzymes and hydrogen ions, which work to dissolve the mineralized bone matrix. Once the OCs have completed the resorption phase, there occurs the reversal phase, during which monocytes appear on the bone surface, and start preparing for the subsequent activity of the OBs. These cells also provide the signals needed to recruit and to differentiate the OB progenitors. Once the population of OBs has been activated, they start to synthesize new bone mineral matrix until the resorbed bone has been completely replaced by new bone. When the osteoblastic deposition is completed, these cells can encounter three fates: they may flatten and become lining cells on new bone surface, they may become bone cells called osteocytes (OCys), or they may undergo apoptosis. OCys are completely included within the bone matrix in bone structures called "lacunae". They are extremely important because they are the cells that transmit the signals regarding bone stress [13]. In a healthy skeleton, the bone remodeling process ensures a balance between bone resorption and bone formation. Alteration of this balance leads to bone diseases. The main example of a bone disease closely linked to an imbalance in the bone remodeling process is osteoporosis, the most common age-related metabolic bone disorder in developed societies. Osteoporosis is characterized by excessive bone resorption that is not followed by new bone formation. This results in a loss of bone mineral mass, which weakens the skeleton and increases the fracture risk [14]. So, bone remodeling is a complex and organized process whose complex regulation depends on the action of local, autocrine/paracrine, and systemic endocrine regulatory systems [15]. OC maturation and activation are under paracrine control exerted by several factors such as the receptor activator of NFkB ligand (RANKL), osteoprotegerin (OPG), macrophage colony-stimulating factor (M-CSF), and by interleukin-6, which are also derived from the OBs and their progenitors. It is the interaction between RANKL and its receptor RANK on OC progenitors that results in activation, differentiation, and fusion of activated hematopoietic cells to generate active OCs. The effects of RANKL are completely blocked by OPG, a soluble dimeric glycoprotein and member of the tumor necrosis factor (TNF) receptor family, produced by OBs and stromal cells, which acts as an antagonist of RANK. Acting in this way, OPG is a physiologically fundamental regulator of OC activity [16]. Also, OBs are locally regulated by bone morphogenetic proteins and by Wingless-related integration site (Wnts). The binding between RANKL and RANK has also been described to promote osteoclastogenesis and the activation of OCs. It has also been reported that during bone resorption, activated OCs release TGF-b1, which in turn triggers the differentiation and activation of OB precursors.

At the same time, high levels of TGF-b1 in the microenvironment are known to induce inhibition of osteoclastic activity [17].

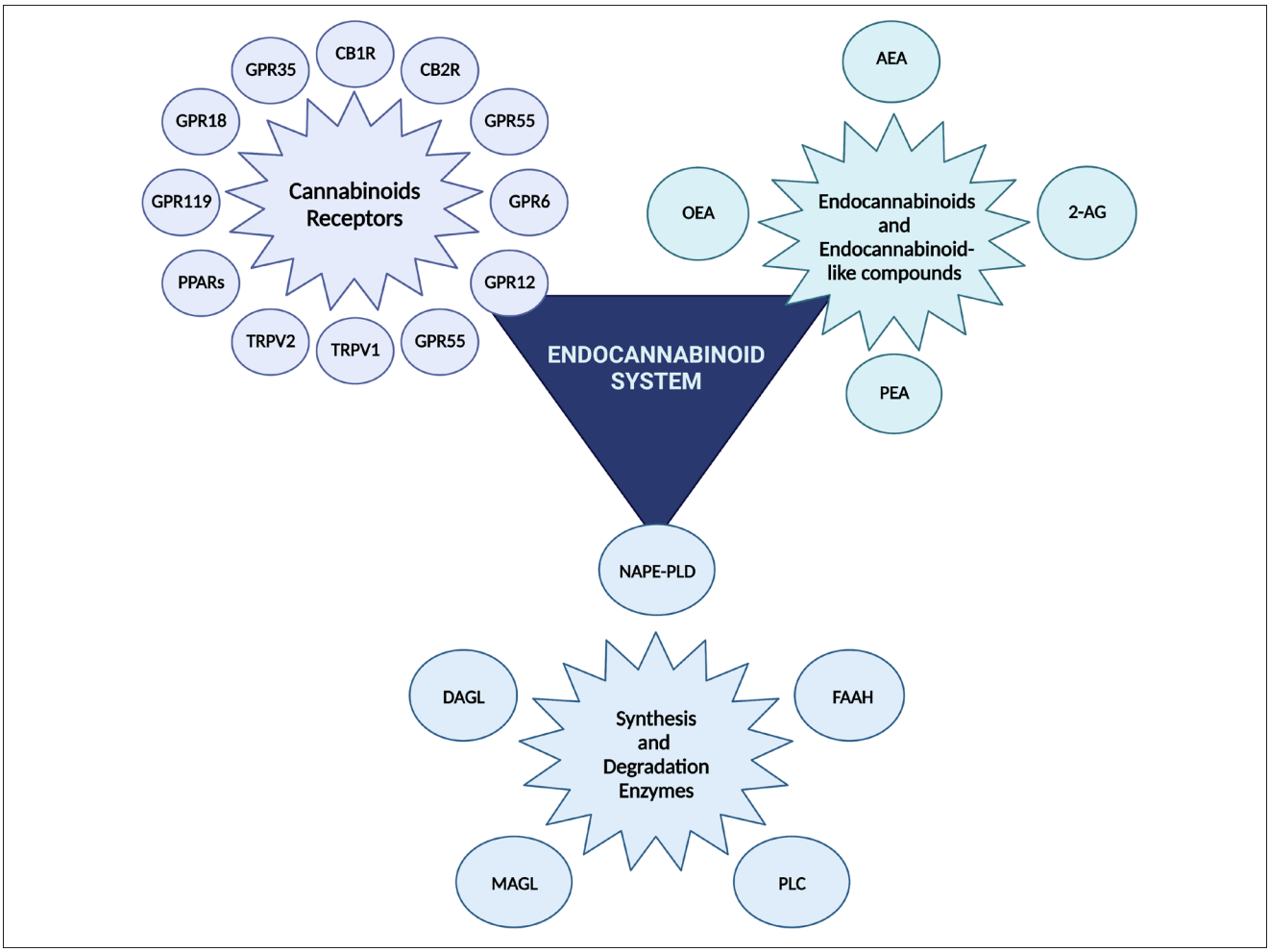
Bone remodeling is also under systemic control by several gonadal hormones, whose depletion in females and males leads to bone loss [18], and by insulin-like growth factor 1 [19], calcitonin [20], and parathyroid hormone (PTH). PTH is the most important regulator of calcium homeostasis and is responsible for maintaining serum calcium concentrations, stimulating bone resorption, and increasing renal calcitriol production and renal calcium resorption. In recent years, PTH has also been shown to be able to augment bone formation [21]. Finally, bone remodeling is also under the control of the central nervous system through hypothalamic leptin and the neuropeptide Y [22].

The endocannabinoid system at a glance

The ES is composed of several components that include (Figure 1):

a) endogenous cannabinoids, or endocannabinoids (ECBs) (i.e., arachydonoyletanolamide, (anandamide, AEA) and 2-arachydonoylglycerol (2-AG);
b) endocannabinoid-like compounds (i.e., N-palmitoylethanolamine (PEA) and N-oleoylethanolamine (OEA);
c) cannabinoids receptors, including the two main cannabinoid receptors, type 1 (CB1R) and type 2 (CB2R), which are both G protein coupled receptors;
d) the transient receptor potential (TRP) superfamily of cation channels, in particular vanilloid receptor-1 (TRPV1) and vanilloid receptor-2 (TRPV2);
e) the enzymes responsible for ECB synthesis, such as N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) for AEA and phospholipase C (PLC) followed by the activity of two selective DAG lipases (i.e., DAGLa and DAGLb) for synthesizing 2-AG;
f) the enzymes able to inactivate AEA and 2-AG, like fatty acid hydrolase (FAAH) and the N-acylethanolamine acid amidase (NAAA), which are both able to hydrolyze AEA into arachidonic-acid and ethanolamine, and the monoacylglycerol lipase (MAGL), which is the principal hydrolase of 2-AG, which can also be hydrolyzed by FAAH;
g) the transporters of ECBs and of PCBs, like the exosomes, adiposomes and endocannabinoid membrane transporter (EMT);
h) other receptors that are able to bind ECBs but have been discovered only in recent years and for this reason are called "orphan receptors" (i.e., peroxisome proliferator activated receptors (PPARs) and some G-protein coupled receptors (i.e., GPR35, GPR55, GPR18, GPR12 and GPR119).

Figure 1 Endocannabinoid system components (created with biorender.com).



As regards to the receptor components of the ES, several studies have been carried out in recent decades to understand both their localization and the several biological effects of ECBs and of PCBs on different tissues, since both exert these effects exclusively through binding to cannabinoid receptors. In addition, the binding of these molecules induces the activation of receptors that trigger a cascade of different signaling pathways. For example, CB1R and CB2R are receptors that couple to Gi/o proteins and go on to inactivate adenylate cyclase, causing a reduction in intracellular cyclic adenosine monophosphate (cAMP) levels. It has also been observed that cannabinoid receptors can act positively or negatively on other intracellular signaling pathways, too, such as MAP kinases. Nevertheless, not all the signaling pathways through which the ES receptors act are known.

As reported in Table I, different ECBs, PCBs, and synthetic cannabinoids can bind to cannabinoid receptors.

Returning to the localization of the ES components, in recent years it has been demonstrated that these components are expressed in bone and in bone cells (i.e., OBs, OCs and OCys), suggesting that the ES, ECBs and PCBs could play an important role in bone homeostasis and in the bone remodeling process, too. Not only, the presence of the ES has also been observed in synovial tissues.

Table I Cannabinoid receptor ligands

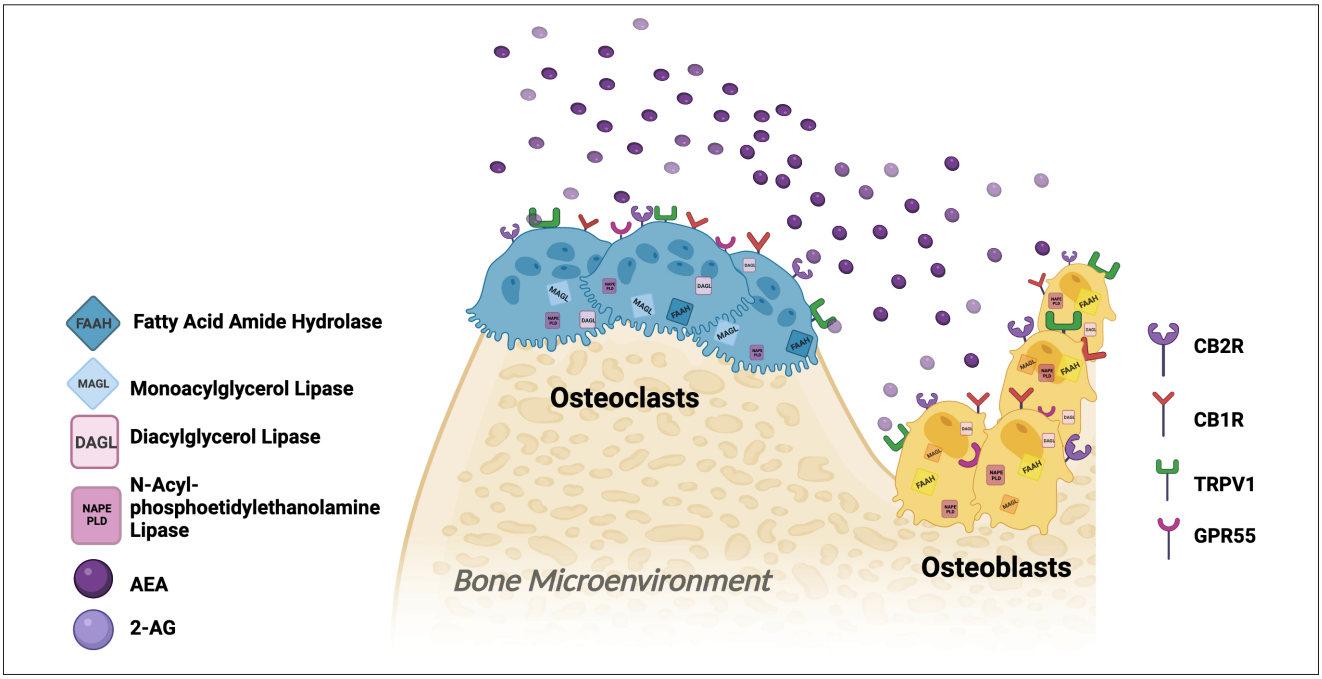
CANNABINOIDS	RECEPTORS
Anandamide (AEA)	CB1R/CB2R/TRPV1/GPR55
2-AG	GPR55/CB1R/CB2R
Cannabidiol (CBD)	CB1R/CB2R/GPR55
D9-THC	CB1R/CB2R/GPR55
WIN55,212	CB1R/CB2R
HU308	CB2R
AM630	CB1R/CB2R/GPR55
JWH133	CB2R/CB1R
AM251	CB1R/CB2R/GPR55
CP55,940	CB1R/CB2R
O-1602	GPR55
Lysophosphatidyl inositol	GPR55
JWH015	CB2R

The endocannabinoid system in the skeletal system

Evidence has been collected over the past ten years regarding the presence of the ES in bone cells and in chondrocytes [23,24].

Bone cells (i.e., OCs OBs) not only express endocannabinoid receptors (i.e., CB1R, CB2R and TRPV1) on their cell membranes, but also possess all the ES machinery for the biosynthesis and degradation of ECBs (Figure 2), so they can synthesize and secrete ECBs according to their maturation and differentiation stage, and work as a regulator in the process of bone remodeling [25]. Osteoblasts and osteoclasts present on the cell membrane of cannabinoid receptors (i.e., CB1R, CB2R, TRPV1 and GPR55) contain enzymes for endocannabinoid synthesis (i.e., NAPE-PLD and DAGL) and degradation (i.e., FAAH and MAGL). As the role of the ES and all its components in bone has begun to be described, the involvement of the ES in joint tissues, too, has started to be investigated. Recent studies have reported the presence of endocannabinoid recep-

Figure 1 Endocannabinoid system components (created with biorender.com).



tors (i.e., CB1R, CB2R, GPR55, GPR18, TRPV1) and also the peroxisome proliferator-activated receptors alpha and gamma (PPARα and PPARγ) on the cell surface of chondrocytes and in particular of osteoarthritic chondrocytes [24]. All this evidence provides important insights into the regulation of bone metabolism and how this relates to joint and bone diseases.

Cannabinoids and evidence of possible modulation of osteoclast function and bone resorption

The presence and activity of the ES have been demonstrated in OC cultures *in vitro*. CB1R, CB2R and GPR55 have all been shown to play important roles in the regulation of OC activity and thus in bone resorption. Idris *et al*., through detailed micro-computed tomography and scanning of bone and histomorphometric analysis of bone formation and resorption, demonstrated that mice deficient in CB1R show a loss of OCs and a reduction in bone resorption, leading to the idea that endocannabinoid receptors play an important role in the regulation of peak bone mass. They also conducted an *in vitro* study on murine OCs demonstrating that inverse agonists/antagonists of CB1R, AM251, and SR141716A are able both to induce OC apoptosis and to inhibit OC differentiation *in vitro*, while activation of CB1R led to differentiation and activation of OCs with consequent activation of the bone resorption process [23]. Whyte *et al*., conducting an *in vitro* study in human OCs derived from monocytes isolated from healthy donors, demonstrated that CB1R and CB2R are present on undifferentiated monocytes and in mature OCs, where CB1R is expressed throughout the differentiation process, while CB2R decreased at the end of the monocyte's differentiation process but remained present on the cell membrane of mature OCs. In addition, Ofek *et al*. reported that CB2R-deficient mice developed osteoporosis with increasing age with a reported increased bone turnover. They also found that HU308, a selective agonist of CB2R, inhibited RANKL-induced OC formation in wild-type bone marrow cultures and observed no inhibitory effects of HU308 in OC cultures isolated from $CBR2^{-/-}$ mice, demonstrating that this molecule acts through CB2R [26]. On the contrary, Idris *et al*., in their work on CB2R in OCs, demonstrated that HU308, AEA, 2-AG, and JWH133, another synthetic CB2R agonist, all stimulate M-CSF- and RANKL-induced OC formation over the concentration range 1-1000nM, while AM630, a CB2R-selective antagonist had an inhibitory effect on OC formation [23]. In another study, the same authors demonstrated that bone marrow cells isolated from $CB2R^{-/-}$ mice produce fewer mature OCs in response to RANKL than wild-type control, and that $CB2R^{-/-}$ mice were partially protected from ovariectomy-induced bone resorption and bone loss as compared with wild-type littermates [27].

In the same period Rossi *et al.* reported that AM630 at a high concentration (10mM) stimulated OC formation and activation in human OC cultures [28], which is the opposite of what Idris *et al*. reported when treating murine OC cultures with AM630. These conflicting results are probably related to species-related differences in responsiveness to AM630. Also, studies on the role of the ES and cannabinoids on bone metabolism have been performed on human bone cell in vitro models.

Ridge *et al*. and Whyte *et al*. in their study on human *in vitro* OC models found that AEA and 2-AG were both able to stimulate bone resorption by human OCs [29]. Schuehley *et al.* introduced a new class of highly CB2R ligands that strongly inhibited RANKL-osteoclastogenesis in both murine and human OC cultures [30]. In the same period Lunn *et al*., studying Sch.036 *in vivo* in arthritic mice, demonstrated that this new highly CB2R ligand prevented bone loss in this model, suggesting an important role also in joint tissue [31].

Finally, Whyte *et al.* demonstrated that adding AEA directly to mature OCs was able to stimulate the OC polarization and resorption and that this effect was reversed by adding CB2R and CB1R antagonists [29].

Other studies have also reported that the GPR55 receptor is able to regulate OC activity and bone resorption. Whyte *et al*., in their 2009 study, observed that L-a-lysophosphatidylinositol and O-1602, two GPR55 agonists, were both able to inhibit OC formation from isolated bone marrow macrophages *in vitro*; nevertheless, the GPR55 antagonist CBD was reported to increase OC formation *in vitro* but to inhibit resorptive activity. In line with these observations, male mice with targeted inactivation of GPR55 ($GPR55^{-/-}$) showed increased numbers of OCs *in vivo*, but these OCs seemed unable to resorb bone, since an increment of trabecular bone mass was observed. On the contrary $GPR55^{-/-}$ female mice were found to have few OCs but an increased amount of unreserved growth plate cartilage [32]. Further study on wild-type mice revealed that CBD can reduce the level of a specific biochemical marker of bone resorption. All these reported observations on the role of GPR55 in osteoclastogenesis and bone resorption suggest that activation of GPR55 inhibits OC formation, but reduces OC resorption activity, while inhibition of GPR55 seems to increase OC formation and reduce the ability of OCs to resorb bone. Finally, in recent decades some studies have started to report that PPARγ could also be implicated in the regulation of OC differentiation [33].

In summary, all previously described receptors can regulate osteoclastogenesis and bone resorption. As we have reported, there are conflicting results regarding the role of CB2R in the regulation of OC differentiation *in vitro* and further research will therefore be required to investigate and try to completely clarify the role of the ES and of cannabinoids in regulating OC formation, differentiation, and function.

Cannabinoids and evidence of their possible modulation of osteoblast function and bone formation

In 2009 Idris *et al.* reported that bone marrow stromal cells isolated from CB1R-deficient mice tended to differentiate into adipocytes rather than OBs, and they observed that this effect could be reproduced by treating OB wild-type cultures with the CB1R-selective antagonist/inverse agonist AM251. At the same time, they observed that AM251 was able to block the stimulatory effect of CP55,940, a synthetic cannabinoid agonist, on bone nodule formation *in vitro*. Hence, Idris *et al*., in their signaling studies regarding the effects of the blockade by

AM251 in OBs and in preadipocytes, observed that this blockade induced up-regulation of cAMP, inhibition of the expression of *RUNX2*, and increased expression of the adipocyte-specific transcription factor *PPARγ* [34]. Other studies carried out by Tam *et al*. reported that bone formation rate and mineral apposition were reduced in young CB1R-deficient mice, confirming that CB1R can play a role in the regulation of the bone formation process [35]. On the contrary, other studies reported involvement of CB1R in glucocorticoid-induced bone loss. The blockade of CB1R seemed to attenuate the glucocorticoid-induced dysfunction in OBs, significantly reducing bone loss and abrogating bone marrow adiposity [36]. As reported for OCs, OBs also express CB2R on their cell membrane and several studies focusing on this receptor have been conducted in recent years to clarify its role in bone formation. CB2R-selective agonists (i.e., HU308, JWH133 and JWH015) were all found to be inducers of bone nodule formation in bone marrow stromal cells *in vitro*, and the same effects were observed when treating these cell cultures with non-selective agonists like AEA, 2-AG, WIN-55,212 and CP55,940 [26,37].

The role of CB2R in bone formation has also been shown by the fact that bone marrow stromal cells isolated from CB2R-deficient mice present a reduced capacity to differentiate into OBs compared with the same cells isolated from wild-type mice [26]. Rossi *et al*. subsequently investigated the role of CB2R and TRPV1 in human OB cultures obtained from healthy donors. They described all the ES machinery in these cells and showed that their stimulation by synthetic agonists was able to regulate the synthesis of osteogenic markers produced by OBs. They also reported that treatment of cultures with RTX, a selective agonist, decreased bone matrix deposition, reducing the expression of all the OB marker genes such as *RUNX2*, *OPG*, and *ALP*. At the same time, by treating OB cultures with JWH133, they were able to induce an increment in the expression of bone apposition markers. It has also been demonstrated that both CB2R and TRPV1 selective agonists are able to increase the production of RANKL by OBs, which is a crucial and fundamental factor for maturation of OCs. Studying another cell model of OBs, Hutchins *et al*. observed an increase in the mRNA expression of *NAPE-PLD*, *FAAH* and *CB2R* during OB maturation [38]. Kostrzewa *et al*., using the same OB model described by Hutchins *et al*., demonstrated that 2-AG shows a peak in pre-osteoblastic cells at the beginning of the differentiation process and a decline during the entire process, suggesting a different role of ES components in different moments of the life of the OB [39]. Finally, GPR55 also seems to be involved in bone formation but to date, too few studies have been performed to establish whether it may play an important role in bone tissue formation, although it has been found to be expressed in both human and mouse OBs.

Cannabinoids in osteoporosis

Osteoporosis is a common age-related disease, which is characterized by progressive bone loss that ultimately results in high morbidity and mortality related to the associated increased fragility fracture risk.

Cannabinoids and the ES have been described to be important regulatory molecules affecting peak bone mass, bone turnover, and age-related bone loss. Even though there is a clear correlation between the ES and bone, as reported in the previous paragraphs, the complexity of the interaction between the components of the ES and bone still needs to be unraveled through *in vivo* and *in vitro* studies. In recent years, several studies in mouse models have revealed how the cannabinoid receptors are involved in the regulation of bone mass. For example, Ofek *et al*. tested the synthetic specific CB2R agonist, namely HU-308, in a mouse model of osteoporosis induced by ovariectomy, observing that the treatment with HU-308 led to a reduction in bone loss, by reducing OC activity and enhancing OB-stimulated bone apposition [26,40]. At the same time researchers are trying to understand the correlation between mutations in the *CB2R* gene and osteoporosis, demonstrating that there are some genetic variations at the loci encoding cannabinoid receptors which might be associated with osteoporosis. In support of this hypothesis, Karfak *et al*., in a 2005 study, observed that 26 single-nucleotide polymorphisms (SNPs) in the *CB2R* gene seemed to be correlated with a significantly lower spinal bone mineral density (BMD) compared with what was observed in osteoporotic patients who did not present these genetic alterations [41]. In a study evaluating the correlation between *CB2R* SNPs and OP, Yamada *et al*. confirmed that the SNP rs2501431, considered one of the most promising by Karsak *et al*., was in fact associated with a significantly lower BMD at the distal radius, lumbar spine and femoral neck compared with control population, as also observed in two other clinical studies [42,43]. At the same time, Richards *et al*. found no significant correlation with spine BMD or fractures and the observed 33 SNPS in the *CB2R* gene.

Oher researchers also evaluated the correlation between genetic defects in the *CB1R* gene and osteoporosis, but to date no variants in this gene have shown a significant association with OP [44-46]. In recent years it has been reported that GPR55 receptor agonists promote bone loss and a recent study on an *in vitro* model of OCs isolated from patients affected by osteoporosis suggested that a desensitization by fatty acid amides, and at the same time TRPV1 agonist-induced overexpression of the CB2 receptor, can be critical to reduce calcium entry into OCs, leading to over-activation of cells and consequently an increase of bone resorption and the final bone loss [47]. The latest evidence has also shown that CB2R agonists can increase bone mass by enhancing the number and activity of OBs, inhibiting the proliferation of OCs [48]. Therefore, these latest results indicate that CBR agonists could be useful for the prevention and treatment of osteoporosis.

Cannabinoids in joint disease

Osteoarthritis is the most common musculoskeletal disease, characterized by persistent or intermittent pain affecting joint tissues. The origin of this pain is still unclear, but there are several hypotheses, such as that the pain could be driven by an inflammatory process, nerve damage, or damage to joint tissues [49,50]. Current treatment options may not be optimal for

all patients, and long-term use of some drugs has the potential for serious unwanted side effects. In view of the therapeutic properties of cannabinoids in pain relief and the fact that the components of the ES have been described to be present also in joint tissues, several studies have started to evaluate not only the possible role of PCBs as a natural analgesic for the development of new therapies against pain, but also how the components of the ES could be involved in the pathogenesis of osteoarthritis, becoming targets for the development of new therapies. A main phenomenon in osteoarthritis is degeneration of the articular cartilage, mediated by complex interactions of proinflammatory cytokines including interleukin-1 (IL-1), inflammatory mediators, and proteases. In recent decades it has been reported that CBs are able to prevent the IL-1-induced disruption of collagen and proteoglycan, suggesting that these molecules can play a protective role in cartilage protection.

Consequently, it has been reported that both CB1R and CB2R are expressed in synovial tissue from patients with osteoarthritis and rheumatoid arthritis and that these produce important physiological effects such as reducing arthritis inflammation and alleviating arthritis-associated pain symptoms [51].

Richardson *et al*. also observed that AEA and 2-AG are detectable in synovial fluids. In accordance with previously collected data, they demonstrated that activation of CB1R and CB2R was blocked by synthetic CB1R and CB2R antagonists, suggesting that the receptors of both cannabinoids may possibly play a role in regulating synovial cell function [52]. A pre-clinical *in vivo* study carried out by Malfait at al. showed that CBD at low doses inhibited the development of collagen-induced arthritis in mice, whereas high doses were less effective [53]. They also reported that CBD could reduce joint damage. Consequently, in their subsequent *in vitro* study they showed that synovial cells explanted from mice treated with CBD released less TNF than synovial cells explanted from control mice. These data demonstrated that CBD has an anti-inflammatory effect, but its mechanism of action remains unclear. Another study conducted by Sumarwilla *et al*. reported that a novel synthetic cannabinoid, HU-320, could be used to treat arthritis in mice thanks to its anti-inflammatory and immunosuppressive properties without showing any psychoactive effect [54].

In accordance with the previous data, other studies have revealed that CBD also has immunomodulatory effects [55,56], and that CB2R seems to be a future therapeutic target in inflammatory arthritis as reported by Lunn *et al*. [31].

Moreover, in recent years, the proinflammatory cytokines responsible for cartilage damage in OA have been found to be produced also by OBs, and it has been shown that high levels of these cytokines in bones and joints induce acute pain, cartilage loss, and eventually joint dysfunction [57-59]. Therefore, acting on the release of the proinflammatory cytokines by OBs seems to represent a good strategy for preventing and treating OA. Yang *et al*. observed that THC inhibited the release of several proinflammatory cytokines from OBs [60]. In recent years, Dunn *et al*. also demonstrated that synthetic designed CBs bind to cannabinoids receptors, leading to inhibition of the catabolic and pain pathways, which are characteristic of arthritic joints, without causing any psychoactive effect, thus suggesting that they may have therapeutic potential for arthritis [24].

Nevertheless, more studies are necessary to clearly understand the role of the ES in joint tissues and their involvement in the pathogenesis of OA, so that the potential usefulness of cannabinoids in OA treatment can be better evaluated.

Conclusions and future directions

The evidence here reported demonstrates that the ES plays an important role in bone remodeling and in regulating bone mass, and that it is also involved in inflammatory processes. This evidence has therefore led to the suggestion that pharmacological modulation of the ES could represent a possible treatment for pathological conditions characterized by altered bone cells and synovial cell activity. Today, a large variety of ES modulators is available, in addition to PCBs. Nevertheless, further *in vitro* and *in vivo* studies are needed to delineate their pharmacokinetic properties, as well as the safety and toxicity profiles not only of synthetic but also of plant-derived ligands of the cannabinoid receptors. As has been reported in this review, while the ES clearly plays a role in bone and joint tissues, further *in vivo* and *in vitro* studies are needed also to understand several aspects of ES mechanisms, for example which endocannabinoids production undergo regulation in bone, and what signaling pathways are used by ES receptors to regulate osteoblast, osteoclast, and synovial cell activity. As herein described, CB1R is known to be able to regulate osteoblast and mesenchymal stem cell differentiation through a cAMP pathway, but we still do not know what mechanism regulates osteoclast activity, and nothing is known about the release of TNF by synovial cells. In conclusion, although there is much scientific evidence that the ES may be a target for the development of therapies for the treatment of bone and joint diseases, making the ES and all its ligands (i.e., PCBs, ECBs, and synthetic cannabinoids) allies in the treatment of bone and joint diseases, many questions remain. Researchers in the coming years will therefore face the task not only of solving them, but also of understanding, based on what has been observed so far, whether the natural components of *Cannabis sativa* may really be considered new natural pharmacological agents in the treatment of osteoporosis and osteoarthritis, as well as valuable allies in the treatment of other diseases affecting these two tissues.

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