The Cannabinoids Effect on Bone Formation and Bone Healing

Bitya Raphael-Mizrahi[1] · Yankel Gabet[1]

Abstract

Purpose of Review Here, we overview the latest findings from studies investigating the skeletal endocannabinoid (EC) system and its involvement in bone formation and resorption.

Recent Findings The endocannabinoid system consists of endogenous ligands, receptors, and enzymes. The main cannabinoids found in the cannabis plant are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Cannabinoid receptors CB1 and CB2 are expressed in bone and regulate bone homeostasis in rodents and humans. CBD treatment was shown to enhance fracture healing in rats. Recent studies in mice indicate that strain, age, and sex differences dictate the skeletal outcome of the EC activation.

Summary CBD treatment was shown to enhance bone healing, but needs validation in clinical trials. While research shows that EC activity protects against bone loss, studies on CB1 and CB2 agonists in bone regeneration models are lacking. Whether modulating the EC system would affect bone repair remains therefore an open question worth investigating.

Keywords Skeleton · Bone · Osteoporosis · Fracture · Cannabis · Cannabinoid · Endocannabinoid · CBD · CB1 receptor · CB2 receptor

Introduction

A variety of therapies including opioids are frequently used to manage severe pain in both cancer and non-cancer patients. Opioids were recently found to inhibit healthy bone remodeling and promote bone loss and fractures [1, 2]. While opioid therapy maybe the most potent analgesic option for cancer patients, it is widely known that patients may develop a life-long dependence for this narcotic substance. Thus, it is important to replace opioid treatment with an alternative pain reliving remedy with no deleterious effects and perhaps even with beneficial actions on bone homeostasis and healing.

The identification of the psychoactive ingredient of cannabis/marijuana, Δ^9-tetrahydrocannabinol, THC [3], leads to the discovery of the endocannabinoid (EC) system. The EC system is a complicated endocrine system consists of ligands, receptors, and biosynthesizing and biodegrading enzymes [4]. The endocannabinoid main endogenous ligands are N-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) [5, 6] that are hydrolyzed by fatty acid amid hydrolase (FAAH) and monoacylglycerol lipase (MAGL) respectfully [7]. The main cannabinoid receptors are CB1 and CB2; both are G protein–coupled receptor (GPCR) class A, seven transmembrane domain [8]. While these receptors are expressed in low levels in a wide range of tissues, high levels of CB1 expression are found in the central and peripheral nervous system and high levels of CB2 expression are found in the skeletal and immune system [9, 10]. In fact, CB1 is one of the most abundant GPCR expressed in the nervous system [11, 12].

The discovery of the skeletal EC system in 2005 revealed its dominant role influencing bone remodeling in health and disease [13, 14]. The EC system effect on skeletal biology was implied from a number of interesting studies. First, it was indicated that leptin negatively regulates bone formation, bone mass, and central production of 2-AG [15]. Secondly, findings show that bone formation and central production of 2-AG are increased in cases of traumatic brain injury [16–18]. These skeletal endocannabinoid connections lead to many more studies investigating the skeletal EC system and the EC activity in bone throughout life. Indeed, the EC system has an important role in the regulation of bone mass and skeletal remodeling in animals [19, 20] and humans [21].

This article is part of the Topical Collection on *Bone and Joint Pain*

✉ Bitya Raphael-Mizrahi
bityar@tau.ac.il

[1] Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, 69978 Tel Aviv, Israel

THC and cannabidiol (CBD) are the main pharmacological active compounds synthesized in cannabis plants. The affinity in which THC binds to CB1 and CB2 is much higher than the affinity of the EC, AEA, and 2-AG [6, 8, 22]. Despite its resemblance to THC at the molecular level, CBD is a weak antagonist at both CB1 and CB2 [23, 24]. The mechanism of actions of CBD is not well understood, yet, it is well accepted that unlike THC, CBD is not psychoactive [25]. In this review, we will focus on the skeletal effects of cannabinoids that are also well-established analgesics. To date, there is a lack of solid evidence on the potential effect of cannabis on bone strength and bone healing. Two groups published conflicted findings on the link between cannabis use and bone mineral density in humans. One study linked excessive cannabis use with low BMD [26•], while the other showed no association between cannabis use and bone health [27•]. Because of the critical differences in the molecular composition of different cannabis strains, and the plethora of synthetic agonists, we will summarize here the specific roles of CB1 and CB2, as well as CBD in bone formation and fracture healing.

Bone EC System

It is well established that the main EC ligands, AEA and 2-AG, are present in the blood circulation [28]. However, the concentrations of these endocannabinoids in the bone and brain tissue are significantly higher, suggesting a local EC production within the bones [19, 29]. Indeed, both osteoblasts (bone forming cells, OB) and osteoclasts (bone resorbing cells, OC) produce AEA and 2-AG in vitro [30]. Moreover, OB, OC, osteocytes (bone cells derived from OB), and chondrocytes (cartilage cells) express both the CB1 and CB2 receptors [30–34]. In vitro activation of CB2 increases osteoblast proliferation [34] and reduces osteoclast numbers [35]. The bone-related CB1 receptor is expressed in the skeletal sympathetic neurons and is located in the neuron terminals, in proximity to the bone cells, negatively regulating norepinephrine production and/or release [30].

Plant-derived cannabinoids, synthetic cannabinoids, and the EC agonists AEA and 2-AG bind to CB1 and CB2 at different binding affinity and selectivity. The relative activation of CB1 versus CB2 in each cell type will dictate the overall skeletal outcome [36, 37]. For the sake of clarity, we will discriminate between the effect of CB1 and CB2 activation reported using genetically modified animal models. Studies on CB1$^{-/-}$ mice and CB2$^{-/-}$ mice indicated that both CB1 and CB2 have a skeletal role [10, 30, 33, 34, 38–40]. These effects are dependent on other factors such as genetic background, sex, age, and hormonal status.

CB1

The skeletal effect of CB1 was found to be age- and strain-dependent [14, 20, 30, 32, 33, 41]. In early reports, findings showed that CB1$^{-/-}$ female mice on a CD1 (a.k.a. ICR) background (CB1$^{-/-}$/CD1) displayed a high peak bone mass and are protected against ovariectomy (OVX)-induced bone loss [14]. These effects were related to the osteoclasts insufficiency in the CB1$^{-/-}$/CD1 mice. However, aged CB1$^{-/-}$/CD1 mice showed an increased age-related bone loss, partially attributed to impaired osteoblast proliferation and differentiation [33]. This is in agreement with a report in rats, showing that CB1 antagonists increased bone mass in young animals and aggravated osteoporotic bone loss in older animals [41]. Together, these reports suggest that CB1 impairs bone accrual in young but has bone protective actions in aged rodents. Interestingly, young CB1$^{-/-}$ mice on a C57BL/6J background (CB1$^{-/-}$/C57BL) displayed a low peak bone mass [32] in both young and aged animals. This suggests a CB1 strain–dependent phenotype in young but not in aged animals, where CB1-deprivation displayed a reduction in bone formation in all strains [14, 30, 33, 39, 41]. CB1 has also been attributed a role in the stimulation of bone formation following head trauma. In mouse models, mild traumatic brain injury resulted in increased bone formation mediated by CB1 in both the calvaria and the femur [32, 42].

Whether the role of CB1 in bone also has a sex-bias remains unclear. Both male and female CB1$^{-/-}$/CD1 showed similar phenotypes at all ages [33]. However, studies on CB1$^{-/-}$/C57BL reported results from female animals only [14, 30]. Further studies are required to determine whether CB1 has a sex-specific skeletal effect in strains other than CD1.

The mechanism of action of CB1 signaling in bone remodeling is still controversial. As mentioned, expression levels of CB1 in bone cells is very low [10, 14, 38]. Nonetheless, OB CB1 levels increase with age, implying that the upregulation of CB1 is protective against age-related bone loss and osteoporosis [33]. The indirect approach proposes that the CB1 regulation of OB activity is mediated by the negative control on noradrenaline release from sympathetic nerve terminals located near the OB, alleviating the noradrenaline inhibition of OB function and bone formation [30]. Although it is possible that the effect of CB1 is both direct and indirect, still, the exact mechanism of the CB1 action on bone formation is yet to be clarified.

CB2

Studies on CB2 revealed that CB2$^{-/-}$ animals have an age-, strain-, and sex-dependent skeletal phenotype. Young CB2$^{-/-}$ mice on a C57BL/6J background (CB2$^{-/-}$/C57BL) displayed normal peak bone mass followed by an increased age-related

bone loss later in life [10]. The aging male and female $CB2^{-/-}$/C57BL showed an increase in bone resorption and bone formation resulting in a high bone turnover with a negative balance outcome [10, 33, 40], similarly to human postmenopausal osteoporosis [43]. This increased age-related bone loss phenotype was the first spontaneous phenotype reported for $CB2^{-/-}$ mice and is in line with reports from human showing that CB2 polymorphism is associated with osteoporosis and bone strength [21, 44]. Several studies demonstrate that the CB2 skeletal effect is strain-dependent as well. In resemblance to CB1, findings indicate that $CB2^{-/-}$ female mice on CD1 background ($CB2^{-/-}$/CD1) and $CB2^{-/-}$/C57BL have a different skeletal phenotype at young age and similar in aged mice. The young $CB2^{-/-}$/CD1 displayed a high bone mass with slow bone turnover relative to WT controls [45]. However, CB2 knockout in aging CD1 mice resulted in bone loss due to accelerated bone remodeling like the aging $CB2^{-/-}$/C57BL mice. Comprehensive transcriptomic data produced in a following study shows that CB2 deficiency has different effects on the genetic profile between the two strains, suggesting that the CB2 deficiency–related skeletal phenotype is strain-specific [46]. Interestingly, a more recent study showed that a CB1 and CB2 combined deficiency, on CD1 background ($CB1^{-/-}CB2^{-/-}$/CD1), prevented age-related bone loss by inhibiting osteoclast formation. In this study, young $CB1^{-/-}CB2^{-/-}$/CD1 presented a high peak bone mass similar to mice of the same age with a single receptor deficiency, $CB1^{-/-}$/CD1 or $CB2^{-/-}$/CD1. Additionally, aged $CB1^{-/-}CB2^{-/-}$/CD1 were partially protected against age-related bone loss, unlike mice with a single cannabinoid receptor deficiency that have an accelerated age-related bone loss [47•].

Interactions between CB2 signaling and sex hormones are not entirely understood. For instance, CB2 deficiency in the CD1 strain did not show a skeletal effect in male mice [45]. Some studies suggested that $CB2^{-/-}$ female mice are partly protected from osteoporosis in the OVX-induced bone loss model [39, 40]. Other studies showed that stimulation of CB2 signaling with the selective CB2 agonists in OVX mice prevents OVX-induced bone loss [10, 48]. Although these studies were not conducted on the same mouse strains, we may speculate that the skeletal actions of sex hormones depend on the presence of CB2, but the skeletal actions of CB2 activation are independent of sex hormones. On the other hand, evidence shows that estrogens can modulate EC ligands in rats as well as CB2 expression in rats and humans [49, 50]. More studies are required to better understand the interactions of CB2 and sex hormones in skeletal homeostasis.

As in the case of CB1, more research is required to determine the exact actions of CB2 on bone formation and resorption. Separate studies reported that both CB2 agonists and CB2 knockout result in increased bone formation rate [10, 40]. The increased bone turnover rate and bone loss in aging $CB2^{-/-}$ mice suggests that bone resorption is inhibited by CB2 signaling in the trabecular bone and the increased bone formation results from the coupling between osteoclasts and osteoblasts [51]. In the cortical bone also, CB2 agonists stimulated endosteal bone formation [10]. Although the skeletal CB2 effect in vivo may be mediated by non-skeletal cells (e.g., monocytes and must cells that release NO in response to ECs, which may in turn affect bone formation [52, 53]), several in vitro studies on isolated OB cultures indicate that CB2 signaling stimulates OB proliferation, differentiation, and osteogenic activity [10, 30, 34, 40]. While studies are in agreement regarding the direct action of CB2 in OB, studies investigating the direct CB2 effect on murine and human OC have reached contradicting results. Some studies concluded that CB2 signaling increases OC differentiation [14, 39], while others in murine and human OC showed evidence that CB2 signaling inhibits osteoclastogenesis [10, 50, 54]. Although these contradictions are not yet settled, the latter results are in line with all the findings that show increased bone resorption in $CB2^{-/-}$ mice [10, 40].

In light of the strain differences observed in mice, it is of utmost importance to elucidate the role of CB2 in humans. While the CB2 exact pathways remains unclear, a substantial number of studies in different human populations indicate that CB2 is strongly associated with bone mineral density [21, 44, 55–57]. Polymorphism in *CNR2*, caused from a non-conservative missense mutation in the *CNR2* sequence (Gly63Arg), effects CB2 expression and activity and is strongly associated with osteoporosis and bone strength in humans [21, 44]. This exact gene polymorphism is also associated with reduced endocannabinoid-modulation of the immune system and is linked to autoimmunity in human Caucasians [58]. These findings are in agreement with the results from the aforementioned studies in C57BL/6J mice supporting the bone protective activities of CB2.

Apart from the age-related low bone mass phenotype in $CB2^{-/-}$ mice, it is noteworthy to mention that $CB2^{-/-}$ mice also display significantly longer femurs and an increase in length of the vertebral bodies when comparing with $CB1^{-/-}$ or WT mice [31, 59]. This indicates that CB2 has a role in the regulation and attenuation of bone elongation in growing animals as well.

Together, all findings demonstrate that both CB1 and CB2 receptors have different but important roles in the skeletal metabolism. However, we could find no studies on the therapeutic potential of CB1 or CB2 selective agonists/antagonists in models of bone healing and bone regeneration. One study tested THC, a CB1/CB2 agonist with a 16-fold higher affinity for CB1, in fracture healing in rats. In this single report, THC had no significant effect on the structural and biomechanical properties of the fracture callus [60••]. Further studies are warranted to determine the therapeutic potential or possible deleterious effects of CB1/CB2 agonists/antagonists in bone healing and regeneration.

CBD

There is a dearth of studies on the potential use of CBD in bone homeostasis, fracture healing, and regeneration. A recent study tested the effect of CBD on spinal cord injury (SCI)–induced bone loss in rats [61]. In this study, researchers found that CBD administration not only attenuated the sublesional cancellous bone loss but also enhanced the mechanical properties of the femurs in SCI rats. Notably, CBD treatment in SHAM rats had no significant effect [61], suggesting that CBD specifically prevented the bone detrimental effects of SCI. We generated unpublished data confirming CBD has no effect on bone homeostasis in mice. Mice treated with 5 mg/Kg/day of CBD were analyzed at 12 weeks of age and showed no difference in the trabecular (trabecular bone fraction, BV/TV = $6.62\% \pm 2.39$ and $6.92\% \pm 2.60$ in treated vs non-treated mice, $p = 0.94$) and cortical (cortical thickness: 146 ± 12 μm and 146 ± 14 μm in treated vs non-treated mice, $p = 0.97$) femoral bone parameters. On the other hand, CBD may have a therapeutic effect in bone repair. One study investigated the effect of CBD alone or in combination with THC in a fracture healing model in rats [60••]. Their results indicated that CBD alone significantly improved the mechanical properties of the fracture callus although it did not increase its volume or mineral content. Treatment with THC alone and in combination with CBD did not improve but also did not impair bone healing. This CBD-induced increase in callus strength and toughness was associated with the increased expression of procollagen-lysine 2-oxoglutarate 5-dioxygenase (PLOD1), a collagen cross-linking enzyme, in cultured primary osteoblasts treated with CBD [60••]. These results revealed a specific action of CBD in enhancing fracture healing in long bones by enhancing the biomechanical quality of the newly formed bone. A more recent study confirmed that CBD enhances healing and improved biomechanical properties in a rat model of critical-sized defect in long bones [62••]. In this study, CBD improved the migration and osteogenic differentiation of mesenchymal stem cells, leading to improved bone bridging across the defect. Together, these two studies support the idea that CBD offers a promising therapeutic option to enhance bone healing and regeneration, in addition to its known analgesic and anti-inflammatory effects. Yet, more studies are required to validate these findings in other species and better characterize the exact mechanism of action of CBD.

Summary

Since its discovery, the clinical potential of modulating the EC system to improve bone health and regeneration attracted increasing interest in the scientific and pharmaceutical fields. To date, no cannabinoid component was yet approved for treating skeletal disorders and injuries.

The experimental evidence from animals and humans strongly suggests that CB1 and CB2 are bone protective. Studies in mice determined that CB2 activation stimulates bone formation and inhibits bone resorption. A number of studies in humans indicate that CB2 is protective against osteoporosis and may prevent bone fractures. To date, there are no reports on the therapeutic potential of CB1 or CB2 agonists and antagonists in bone healing and regeneration. Whether modulating the EC system would affect bone repair remains therefore an open question that is worth investigating.

The main non-psychoactive component of cannabis, CBD, had no significant effect in normal bone homeostasis but it was found to be beneficial in enhancing healing and recovery following bone injury.

Taken together, there is growing evidence that the EC system holds important roles in skeletal homeostasis throughout life. Cannabinoid based therapies targeting the EC receptors may be used for treating and preventing age-related and hormone deficiency–related bone loss and osteoporosis. Also, the polymorphism in CNR2 can be used as an early diagnostic tool to detect genetic-predisposition to osteoporosis in humans. Further experimental and clinical studies are warranted to establish the putative therapeutic benefit of EC modulators and phyto-cannabinoids for the treatment of skeletal injuries and regeneration.

Compliance with Ethical Standards

Conflict of Interest B.R-M. and Y.G. declare no conflict of interest related to this study.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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