Mini Review

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A collaboration investigating endocannabinoid signalling in brain and bone

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Abstract: Investigations into the cellular and molecular mechanisms underlying the psychoactive effects of cannabis preparations have led to the discovery of the endocannabinoid system. Interest in the central nervous system effects was initially the main focus of the research, but it soon became evident that the endocannabinoid system affects virtually every organ. The research field has therefore experienced a tremendous growth over the last decade and is now truly interdisciplinary. This short review provides a personal account of an interdisciplinary collaboration between Itai Bab from the Hebrew University of Jerusalem and the author. It describes the discovery of the endocannabinoid system in bone and the analysis of its functions. I am summarising the role of CB1 signalling as a modulator of sympathetic inhibition of bone formation. Thus, activation of CB1 receptors on sympathetic nerve terminals in bone, presumably from endocannabinoids released from apposing osteoblasts, reduces the inhibition of bone formation by sympathetic noradrenaline. CB2 receptors on osteoblasts and osteoclasts also modulate the proliferation and functions of these cells. Thus, activation of CB2 stimulates bone formation and represses bone resorption, whereas the genetic disruption of CB2 results in an osteoporosis-like phenotype. This signalling mechanism is clinically relevant, as shown by the association of polymorphisms in the CB2 receptor gene, CNR2, with bone density and osteoporosis. Finally, the review provides a summary of the recently discovered role of endocannabinoid signalling in one elongation. This review will also discuss the benefits of interdisciplinary and international collaborations.

Keywords: cannabinoid receptors; genetics; mouse models.

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It is not the intention of this short review to summarise all studies investigating the role of the endocannabinoid system in bone physiology and skeletal disorders. Rather, it is an attempt to highlight some of the results of the collaboration between Itai Bab and myself over a 12-year period. More comprehensive reviews on the subject area can be found elsewhere [1–6].

It was in the year 2002 when I first received an email from Itai Bab indicating that he would be interested in collaborating with me on the role of endocannabinoid signalling in bone remodelling. This is a life-long process, where bone is removed by osteoclasts and replaced by newly formed bone through the activity of osteoclasts [1, 3–8]. Bone remodelling serves to adapt the skeleton to changing mechanical requirements and also to repair microdamage. In healthy adults, bone resorption and formation are balanced.

Being a neuroscientist, I had studied the involvement of this system in brain functions and animal behaviours using mice lacking the cannabinoid CB1 and CB2 receptors (CB1/−, CB2/−), which my colleagues and I had generated [9–11]. CB1 is most prominently expressed in brain tissues, whereas CB2 is mostly present in peripheral tissues, including immune cells. These knockout mouse models represented instrumental tools to delineate the contribution of both receptors to the pharmacological effects of cannabinoids and the physiology of the endocannabinoid system [12]. They were probably the main reason why Itai Bab contacted me.

Outside the central nervous system (CNS), both receptors have been implicated in the modulation of different pathologies [12], and there was a growing interest in the medicinal use of cannabis preparations. Although scientific and anecdotal evidence suggested that cannabis-based medicines might be beneficial in a wide spectrum of disorders, I did not recall having ever read anything about bone disorders. There was, to the best of my knowledge, no evidence for a skeletal cannabinoid system. Indeed, a quick search on PubMed with “bone” and “cannabinoid” as search terms did not retrieve a single relevant publication.
I was therefore a bit surprised by Itai Bab’s email, and I had some doubts that endocannabinoid signalling would be of relevance to the skeleton. Nevertheless, I took the mail seriously, because Itai Bab was a full professor at the Hebrew University of Jerusalem. I was well aware of the many significant contributions from scientists of this university and had a profound respect for those that I knew personally. Rafael Mechoulam, who had discovered tetrahydrocannabinol (THC) in marijuana as well as the endogenous endocannabinoids, was one of them. I also took the mail seriously, because we had just published a paper showing that CB2 affected the activity of macrophages [11], to which osteoclasts, the bone-resorbing cells, are related. The possibility that Itai might discover a completely novel aspect of endocannabinoid signalling and bone biology was very appealing.

After exchanging a few more emails, we finally met in Bonn. This meeting was the beginning of an extremely fruitful collaboration and of our friendship. Itai had already performed several experiments showing that cannabinoid receptors and fatty acid amid hydrolase are expressed in bone. He also had in vitro data indicating that cannabinoids affect the activity of bone cells. We decided that Itai would investigate the skeletal phenotype of our knockout mice, while we would begin to search for associations between polymorphisms in genes of the endocannabinoid system and osteoporosis. Thus we sent CB1−/− and CB2−/− mice to Jerusalem and contacted Marie Christine de Vernejoul from the Hôpital Lariboisière in Paris, who had assembled a cohort of osteoporosis patients.

Both of these experimental approaches worked extremely well. Within a few months, we obtained a strong support for Itai’s idea that the endocannabinoid system is a major regulator of bone turnover [13]. Expression analysis showed that all components of the endocannabinoid system (receptors and biosynthetic and metabolic enzymes) were present in bone, and endocannabinoid measurements showed that the levels in bone and brain tissues are similar. Most striking, however, was the skeletal phenotype of the knockout mice. Routine handling of CB2−/− mice showed no overt phenotypes, but more detailed experiments that challenged the immune system revealed changes in immune and neuroinflammatory responses [1, 11, 12, 14–18]. Female CB2−/− mice showed a significantly reduced trabecular bone volume density already at an age of 8 weeks [13]. At 52 weeks of age, both sexes showed a markedly lower bone density, with a transition from a plate-like to a rod-like trabecular structure. Concomitant with that was an increased cortical expansion, which resulted from increased diaphyseal and medullary cavity diameters with preserved cortical thickness. The pathology pointed to an increased bone resorption rate in these animals [19]. Indeed, the number of osteoclasts was increased by approximately 40% in CB2−/− females, as were the mineral appositional and bone formation rates. It thus appeared that the deletion of the CB2 receptor resulted in a progressive age-related bone loss, which was similar in many respects to the pathology observed in postmenopausal osteoporosis.

Also exciting was the finding that synthetic CB2 agonist HU-308 attenuated ovariectomy-induced trabecular bone loss and increased cortical thickness, probably due to an increased proliferation of osteoblast progenitors and a repressed osteoclastogenesis [13]. The mitogenic activity of HU-308, or other CB2 agonists, was strictly dependent on CB2 and not observed in bone marrow-derived osteoblasts from CB2−/− mice [20]. The molecular pathway underlying the mitogenic activity of CB2 activation involved an activation of Erk1/2 phosphorylation, followed by the synthesis of Mapkapk2 mRNA and protein. This was followed by a further downstream CREB-dependent transcriptional activation and cyclin D1 expression [20]. Together, these findings strongly suggested a possible use of CB2 agonists in the pharmacotherapy of osteoporosis.

The human genetic studies also provided strong evidence that CB2 signalling is clinically relevant. In a sample of postmenopausal osteoporosis patients and age-matched controls, we found a significant association of polymorphisms in the CNR2 gene, which encodes the CB2 receptor, and Cnr2 haplotypes with the disease phenotype [21]. Importantly, the associated polymorphisms included a missense variant, Gln63Arg, indicating that variations in the functionality of the CB2 receptor contributed to or even caused the phenotype. Subsequent studies not only confirmed the association of the Gln63Arg variant with bone density in ethnically distinct cohorts [22, 23] but also revealed an association with schizophrenia [24]. Molecular studies in transfected CHO cells showed that the reduction of cAMP levels by CB2 agonists was impaired in the Arg63 variant as compared to the Gln63 variant.

Together, these findings suggest a scenario where CB2 activation stimulates osteoblast proliferation and osteoclastogenesis (Figure 1A). A reduced efficacy of CB2 signalling, caused by genetic manipulations in the mouse or natural variations in humans, result in a lower bone density and may lead to osteoporosis. In contrast, activation of CB2 with synthetic agonists stimulates bone formation in animal models of osteoporosis [3–5]. CB2 activation is not associated with the psychotropic effects of cannabinoids, which are attributed to CB1. Thus, selective
CB2 agonists may provide a safe and effective pharmacotherapy of osteoporosis, without unwanted psychotropic effects.

CB1−/− mice were viable but exhibited an increased mortality and a number of behavioural phenotypes [9, 10]. They were almost completely resistant to the psychotropic effects of cannabinoids, indicating that this receptor mediated most of the effects of cannabinoids on the brain. Numerous studies with these mice showed that CB1 signalling was part of an important synaptic feedback mechanism [25–27]. In the absence of CB1, mice showed deficits in learning and memory, as well as alterations in affective behaviours, nociception, hedonic responses, drug reward and other behavioural changes [12, 28]. CB2−/− mice also showed a bone phenotype, but here the situation was more complex [29, 30]. On a C57BL/6 genetic background, CB1−/− mice of both sexes had a substantially reduced bone mass. They showed a reduced bone volume density, a lower density of their trabecular network and reduced diaphyseal and medullary cavity diameters, but no change in cortical thickness. Histomorphometric analysis of these mice revealed a substantially reduced bone formation rate and an increased number of osteoclasts in female mice. In contrast, male CB1−/− mice on a CD1 genetic background displayed a high bone mass phenotype with a higher bone volume density and an increased trabecular thickness. Female CB1−/− animals on a CD1 genetic background showed a normal bone volume density but slightly increased diaphyseal shaft and medullary cavity diameters.

Figure 1: Bone growth and remodelling.

(A) Bone is richly innervated by sympathetic nerve terminals, which release norepinephrine (NE) and inhibit bone formation by activating β2-adrenergic receptors (β2AR) on osteoblasts. CB1 receptors are present on these nerve terminals and are activated by endocannabinoids (eCBs) released from apposed osteoblasts. CB1 signalling inhibits NE release, thus reducing the sympathetic tone and alleviating its inhibitory effects. CB2 receptors are mostly present on osteoblasts and osteoclasts. Activation of CB2 receptors enhances the proliferation of osteoblast progenitors and restrains osteoclastogenesis. Although bone remodeling is stimulated in CB2-deficient mice, there is a net loss of bone mass, which results in an age-related osteoporosis phenotype. (B) Cannabinoid CB1 and CB2 receptors, as well as endocannabinoid synthetic enzymes, are also expressed in the epiphyseal growth cartilage. CB1 is mostly present in the hypertrophic cell layer. CB2, DAGLα and DAGLβ are found in the transitional zone between proliferating and hypertrophic cells. The epiphyseal growth cartilage contains significant levels of 2-AG. Mice lacking CB2 receptors have longer femora and vertebral bodies resulting in a longer stature, whereas stimulation of CB1 restrains bone growth.
The reason for these striking strain differences remains obscure, but some light has been shed on the mechanisms involved in the CB1 modulation of bone remodelling. Unlike CB2, CB1 receptors are not expressed on bone cells. Instead, they were found on sympathetic nerve endings that richly innervate bone [31]. These fibres release norepinephrine, which restrains bone formation and stimulates bone resorption [32, 33], through a mechanism involving the activation of β2-adrenergic receptors on osteoblasts. CB1 activation on sympathetic nerve endings by 2-AG, which is produced from closely apposed osteoblasts, inhibited norepinephrine release, thus alleviating the inhibitory sympathetic tone and stimulating bone formation (Figure 1A). This mechanism is similar to the endocannabinoid inhibition of sympathetic activity in other organs [34–36]. The cannabinoid mechanisms in the neuronal regulation of bone remodelling could be highly relevant in brain diseases associated with bone pathology, such as the low bone mass associated with depression or traumatic brain injury which stimulates bone formation [31]. The skeleton is also innervated by parasympathetic projections, which release acetylcholine and decrease bone resorption [37]. Cannabinoid signalling is thus involved in the CNS regulation of bone remodelling via antagonistic sympathetic and parasympathetic projections. This mechanism is probably clinically relevant in brain disorders with associated bone pathology. These include major depression, which is often accompanied by a reduced bone mass [33, 38, 39], and traumatic brain injury, which is known to stimulate bone formation [31, 40].

The osteoporosis-like phenotype of the CB2−/− mice was also of interest in the context of the role of endocannabinoid signalling in ageing and age-related disorders. Osteoporosis, like dementia, is a major health issue in the ageing population. It has been noted that endocannabinoid signalling in the brain changes during the ageing process. It peaks during adolescence, remains relatively constant during adulthood and declines in old individuals [2, 41]. Mice with mutations in cannabinoid receptors displayed enhanced age-related changes in those tissues where the receptors have prominent physiological functions: CB2 in bone and CB1 in the brain. Young CB1−/− mice showed no cognitive deficits. This is similar to the very minor bone phenotype observed in young CB2−/− mice. However, as CB1−/− mice grew older, they showed a rapid decline of cognitive functions, neuroinflammation and neurodegeneration, which are all hallmarks of pathological brain ageing [28, 42–44].

While investigating the bone volume density of cannabinoid receptor-deficient mice, Itai and his co-workers also observed that femora of CB1−/− and CB2−/− mice were considerably longer at the end of the rapid growth phase compared to wild type animals. Bone growth is determined by the epiphyseal growth cartilage (EGC) [45], which drives bone and consequently body growth by endochondral bone formation. In this process, chondrocyte progenitors differentiate into proliferative and then hypertrophic chondrocytes. The extracellular matrix separating the hypertrophic chondrocytes is then calcified, resorbed by osteoclasts/chondroclasts and replaced by bone. Chondrocytes and osteoblasts have some common regulatory mechanisms [46], and therefore, it was interesting to determine if the endocannabinoid signalling system would be one of these.

As a first step, we investigated the expression of the endocannabinoid system. CB1 is expressed in the hypertrophic cell layer and CB2 in the transitional zone between proliferating and hypertrophic cells. DAGLα and DAGLβ are also expressed in the hypertrophic and transitional cell layers (Figure 1B). Consistent with the expression of these biosynthetic enzymes, we also detected 2-AG at significant levels in the EGC.

Considering the phenotype of knockout mice and the presence of an endocannabinoid system in the EGC, it was interesting to note that several human and animal studies reported that THC exposure during pregnancy restrained foetal growth [47–53]. We therefore wondered if THC would also inhibit growth when administered to young mice at an age of 5–11 weeks. Indeed, we observed a substantial decrease in femoral and vertebrate length in the animal group receiving THC. This effect of THC was maintained in CB2−/− mice but not observed in CB1−/− animals, indicating that CB1, but not CB2, was responsible for the inhibitory effects of THC on bone growth. THC probably acted on hypertrophic chondrocytes, because it reduced the hypertrophic cell zone thickness. Also, THC inhibited EGC chondrocyte hypertrophy in ex vivo cultures. These findings may be of relevance to humans, because recreational use of marijuana is common among teenagers in their skeletal growth phase [54]. Itai submitted the first version of the manuscript reporting these findings [45] but unfortunately became ill during the revision process and passed away before the paper was accepted.

The personal nature of this account also aims at illustrating some of the benefits of interdisciplinary and international collaborations for science and the scientists involved. First, our collaboration shows that it can make sense to cross the boundaries of scientific disciplines. Itai was a dentist specialised in bone biology; I am a biologist specialised in molecular and behavioural genetics. I truly believe that many of our discoveries...
were greatly facilitated by the fact that our experience and our experimental approaches were complementary. The collaboration was initially triggered by a need for specific tools and expertise from the other partner. It then carried on because we were both curious about a scientific topic and because we had developed a personal friendship. Interdisciplinary collaborations such as ours often seem to evolve in a similar manner. They are not frequent but also not uncommon. However, it would not have been possible to cooperate in the way we did without the means to finance it. Fortunately, we were able to obtain several joint grants from the German Israeli Foundation, the Deutsche Forschungsgemeinschaft and the Volkswagen Foundation. Such transnational joint grants are, unfortunately, much more difficult to get for researchers in other countries. We took advantage of the special relationship between Germany and Israel.

I also found that the interactions between our laboratories had an important impact, apart from the direct scientific results. My first visit to Itai’s lab was also my first visit to Israel. I cannot possibly count how often I have come to Israel since and how much time Itai has spent in turn with us. He was a true member of our lab, not only as a collaborator but also as a teacher for our students. Itai’s approach to science, the way he addressed a scientific problem and the way he looked at data, was different from mine. This often led to interesting discussions, which were probably stimulating not only for me but also for our students and co-workers involved. Many of my students and postdocs have also visited Itai’s lab, and, in turn, we have hosted several of his students in Germany. The impact that these interactions and exchanges have on young researchers, on their scientific thinking and their understanding of our countries, cultures and heritage, cannot be overestimated.

A final point I should like to make, even though it may be rather unusual for a scientific review, is the personal impact of our collaboration. Itai and I became friends. Is this important for science? I think it is critically important for several reasons. First of all, it is much easier to collaborate with someone you like and whom you trust. Also, being able to make friends all over the world and meeting them regularly is, at least for the author, one of the most important benefits associated with our profession. Sometimes, as in our case, whole families get involved, thus making the friendship even more rewarding. Could there be anything better than being a scientist in a country with the freedom to pursue a scientific topic of your own choosing and the liberty to interact with colleagues around the world?

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