INTRODUCTION

Osteoporosis is a worldwide health problem with a high prevalence and is a skeletal disorder characterized by bone mass reduction, increased bone fracture risk and potential bone architecture alterations (1). The incidence of osteoporosis is increasing owing to increasing elderly population. Glucocorticoids are widely used for chronic treatment of a variety of diseases as bronchial asthma, collagen and skin diseases. Because of improvements in the outcome of these diseases, the long-term side effects of glucocorticoid treatment on bone remodeling in rats. Sixty four rats were divided into two main groups; group 1 (G1) consisted of 12–14 month old rats and group 2 (G2) consisted of 3–4 month old rats. Each main group subdivided into four subgroups as follows: (NC1) and (NC2), the negative control groups, (MP1) and (MP2), received methylprednisolone (glucocorticoid), (RIM1) and (RIM2), received rimonabant, (MP + RIM1) and (MP + RIM2) received methylprednisolone with rimonabant. There was a significant decrease in bone mineral density (BMD) and bone mineral content (BMC) of the tibia bones together with a decrease in osteoprotegrin (OPG) expression but with a significant increase in receptor activator of nuclear factor kappa B ligand (RANKL) expression in osteoporotic rats. These parameters were reversed with co-administration of rimonabant with methylprednisolone in young rats, though it increased the severity of osteoporosis in older rats. Image analysis technique revealed that there was a significant improvement in cortical bone thickness (CBT) and mean trabecular bone density (TBD) in young group only after rimonabant either alone or with glucocorticoid. CB1 receptors play age related different roles in bone turnover. So, CB1 antagonist can be used to prevent corticosteroid induced osteoporosis in young age but should be avoided in old age.

Key words: osteoporosis, cannabinoids, methylprednisolone, rimonabant, osteoprotegrin, receptor activator of nuclear factor kappa B ligand, osteoporosis, cortical bone thickness, trabecular bone density

EFFECT OF CANNABINOID RECEPTORS 1 MODULATION ON OSTEOPOROSIS IN A RAT MODEL OF DIFFERENT AGES

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Osteoporosis is a common health problem. The endocannabinoid pathway has been implicated as an important regulator of bone turnover. Rimonabant is a potent cannabinoid receptor1 (CB1) receptor antagonist with wide therapeutic use as an antiobesity drug that has been withdrawn due to side effects in the form of depression and suicidal attacks. This study investigated whether glucocorticoid induced bone loss is linked to CB1 signaling and whether modulation of CB1 function affects the deleterious effects of glucocorticoid treatment on bone remodeling in rats. Sixty four rats were divided into two main groups; group 1 (G1) consisted of 12–14 month old rats and group 2 (G2) consisted of 3–4 month old rats. Each main group subdivided into four subgroups as follows: (NC1) and (NC2), the negative control groups, (MP1) and (MP2), received methylprednisolone (glucocorticoid), (RIM1) and (RIM2), received rimonabant, (MP + RIM1) and (MP + RIM2) received methylprednisolone with rimonabant. There was a significant decrease in bone mineral density (BMD) and bone mineral content (BMC) of the tibia bones together with a decrease in osteoprotegrin (OPG) expression but with a significant increase in receptor activator of nuclear factor kappa B ligand (RANKL) expression in osteoporotic rats. These parameters were reversed with co-administration of rimonabant with methylprednisolone in young rats, though it increased the severity of osteoporosis in older rats. Image analysis technique revealed that there was a significant improvement in cortical bone thickness (CBT) and mean trabecular bone density (TBD) in young group only after rimonabant either alone or with glucocorticoid. CB1 receptors play age related different roles in bone turnover. So, CB1 antagonist can be used to prevent corticosteroid induced osteoporosis in young age but should be avoided in old age.

Key words: osteoporosis, cannabinoids, methylprednisolone, rimonabant, osteoprotegrin, receptor activator of nuclear factor kappa B ligand, osteoporosis, cortical bone thickness, trabecular bone density
adipocyte number (15). It has been reported that CB1 signaling affects the glucocorticoid regulation of the hypothalamus function (16). Although no distinct abnormalities in bone development were observed in healthy adult mice deficient in cannabinoid type 2 receptors (CB2), pharmacological blockade of this receptor is effective in suppressing bone loss associated with increased bone turnover (17). Wang et al. demonstrated that skeletal tissue responds to supraphysiologic levels of glucocorticoid with decreased bone formation (18, 19). Rimonabant is a potent CB1 ligand, with 1000-fold affinity for CB1 than CB2. It is a CB1 receptor antagonist in the brain and peripheral organs as bone system with therapeutic use as an antiobesity drug (20).

The present study, investigated the modulation of CB1 function can affect the deleterious effects of glucocorticoid treatment on bone remodeling in skeletally immature and mature rats.

MATERIALS AND METHODS

Experiments were performed according to the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council, Washington, DC, National Academy Press, no. 85-23, revised 1996). All protocols were approved by our Local Committee of Animal Care and Use Committee.

All experimental osteoporosis protocols can be implemented in skeletally immature or mature rats (21). Although rats reach sexual maturity at the age of 2.5 months, their skeleton is considered mature after the age of 10 months (22).

Sixty four male Sprague Dawley rats were obtained from the Animal House Colony of Medical Experimental Research Centre, Mansoura University, Egypt. The animals were housed in plastic cages at room temperature (25 ± 2°C) under a 12 hour dark-light cycle. They were provided with tap water and balanced diet ad libitum and allowed to acclimate for two weeks to housing conditions.

They were divided into two main groups each consisted of 32 rats: group 1 (G1) consisted of skeletally mature rats (12–14 month old, 300–350 g) and group 2 (G2) consisted of skeletally immature rats (3–4 month old, 250–280 g). Each main group were subdivided into four subgroups (each one consisted of 8 rats) as follows: subgroups (NC1) and (NC2), the negative control subgroups in which rats received vehicle injection, subgroups (MP1) and (MP2), were received methylprednisolone sulfoxide (Sigma-Chemical® St. Louis, MO, USA) and then subanorectic dose 50 ug/kg/ intraperitoneally (cayman chemical, Sterling, MD, USA) with 1/2 X photo adaptor , using a 40X objective. The resulting slides were photographed using Olympus® digital camera installed on the Olympus® microscope with 1/2 X photo adaptor, using a 40X objective. The resulting images were saved as TIFF files to be analyzed with Intel® Core I3® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for length and area fraction measurement.

Cortical bone thickness was measured by a free hand line tool which is calibrated against a micrometer slide photographed under the same conditions.

The trabecular bone area was manually extracted with the aid of genius® G-Pen F50® tablet. Total area of the extracted trabecular bone was measured. Then the empty areas were subtracted according to color variation and the area was measured again. Area fraction was calculated in percents. Mean cortical bone thickness (CBT) (µm) and mean trabecular bone density (TBD) (%) were obtained by measuring 5 fields/slide from 5 slides for each rat. The reading of each animal was considered as one variable.

Serum measurements

Serum total calcium (25) and alkaline phosphatase (ALP) activity (26) were measured by colorimetric method (Chremoy, Biochemical Trade, Inc. USA) in Beckman Coulter DU-70 spectrophotometer (Beckman Coulter Inc., CA, USA). Serum osteoprotegerin (OPG), receptor activator of nuclear factor kappa B ligand (RANKL) levels were determined by enzyme linked immunosorvent assay (ELISA) technique using R&D Elisa (Sorin Biomedica, Eti-System, Denlay Instruments Ltd, England) kit as described by O'Brien et al. (27) and Teng et al. (28) respectively. Serum osteocalcin was also measured with osteocalcin (OC) rat ELISA system (Rat Bone Panel3 Millipore, MA, USA) (29).

Real-time PCR analyses of bone osteoprotegrin and receptor activator of nuclear factor kappa B ligand

The metaphyseal portion of the tibia was cut and homogenized using a Mixer Mill MM400 (Retsch, Germany) to isolate the mRNA. Total RNA was isolated using Trizol following the manufacturer's guidelines (Invitrogen, Rockville, MD, USA). The concentration and purity of the RNA were determined by measuring the absorbance at 260 nm and 280 nm. The amount of RANKL and OPG mRNA was determined with ABI Prism 7900HT quantitative real-time PCR (Applied Biosystems, Foster City, CA). The primers were as follows: RANKL (forward, 5'-ACC AGC ATC AAA ATC CCA AG-3', reverse, 5'-TTT GAA AGC CCC AAA GTA CG-3') and OPG (forward, 5'-GTT CTT GCA CAG CTT CAC CA-3', reverse, 5'-AAA CAG CCC AGT GAC CAT TC-3'). PCR amplification was carried out in a 20 µL reaction mixture (2 µL of cDNA and 200 nmol/L primers for OPG and RANKL, respectively, and 1 µL SYBR green). The temperature program was as follows:
Table 1. Effect of CB1 blocker and glucocorticoid on serum measurements of: total calcium level, alkaline phosphatase, osteocalcin, osteoprotegrin and RANKL activities in old rat model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Calcium level</th>
<th>Serum alkaline phosphatase activity</th>
<th>Serum osteocalcin activity</th>
<th>Serum OPG activity</th>
<th>Serum RANKL activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.D.</td>
<td>(mg/dl)</td>
<td>(U/L)</td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
<td>(pg/ml)</td>
</tr>
<tr>
<td>NC1</td>
<td>9.50±2.15</td>
<td>125.98±13.40</td>
<td>2.88±0.59</td>
<td>3.03±0.74</td>
<td>80.60±21.05</td>
</tr>
<tr>
<td>MP1</td>
<td>9.57±1.65</td>
<td>179.86±25.48</td>
<td>1.91±0.41</td>
<td>1.58±0.37</td>
<td>175.84±36.84</td>
</tr>
<tr>
<td>RIM1</td>
<td>9.75±2.87</td>
<td>133.41±24.85</td>
<td>2.75±0.71</td>
<td>2.54±0.50</td>
<td>95.90±22.35</td>
</tr>
<tr>
<td>MP + RIM1</td>
<td>9.86±1.46</td>
<td>189.59±25.44</td>
<td>1.78±0.82</td>
<td>1.14±0.26</td>
<td>181.71±34.56</td>
</tr>
<tr>
<td>P value</td>
<td>0.4</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

All results are expressed as mean ± S.D. Non significant: at P>0.05. Significant: at P<0.05. 1: skeletally mature rats (8 rats/group): NC1 (negative control); MP1 (methyl predinisolone group); RIM1 (rimonabant group); MP + RIM1 (rimonabant + methyl predinisolone group).

P value = ANOVA P value for comparison among old age groups; * = significance between control group and other groups; # = significance between methyl predinisolone group and rimonabant group or rimonabant + methyl predinisolone group; ∝ = significance between rimonabant group and rimonabant + methyl predinisolone group.

Table 2. Effect of CB1 blocker and glucocorticoid on serum measurements of: total calcium level, alkaline phosphatase, osteocalcin, osteoprotegrin and RANKL activities in young rat model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Calcium level</th>
<th>Serum alkaline phosphatase activity</th>
<th>Serum osteocalcin activity</th>
<th>Serum OPG activity</th>
<th>Serum RANKL activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.D.</td>
<td>(mg/dl)</td>
<td>(U/L)</td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
<td>(pg/ml)</td>
</tr>
<tr>
<td>NC2</td>
<td>10.98±2.92</td>
<td>141.32±20.60</td>
<td>4.55±1.24</td>
<td>2.89±0.35</td>
<td>59.18±10.23</td>
</tr>
<tr>
<td>MP2</td>
<td>10.64±1.85</td>
<td>200.18±15.29</td>
<td>3.14±0.97</td>
<td>1.16±0.26</td>
<td>160.54±18.42</td>
</tr>
<tr>
<td>RIM2</td>
<td>10.49±2.07</td>
<td>135.40±19.30</td>
<td>4.40±1.01</td>
<td>4.02±0.69</td>
<td>55.00±10.43</td>
</tr>
<tr>
<td>MP + RIM2</td>
<td>10.69±2.37</td>
<td>129.02±16.88</td>
<td>5.21±1.14</td>
<td>3.51±0.93</td>
<td>100.41±11.80</td>
</tr>
<tr>
<td>P value</td>
<td>0.9</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

All results are expressed as mean ± standard deviation (S.D.). Non significant: at P>0.05. Significant: at P<0.05. 2: skeletally immature rats (8 rats/group): NC2 (negative control); MP2 (methyl predinisolone group); RIM2 (rimonabant group); MP + RIM2 (rimonabant + methyl predinisolone group).

P value = ANOVA P value for comparison among young age groups; * = significance between control group and other groups; # = significance between methyl predinisolone group and rimonabant group or rimonabant + methyl predinisolone group; ∝ = significance between rimonabant group and rimonabant + methyl predinisolone group.

Statistical analysis

The results were statistically analyzed using One-way ANOVA test to evaluate the significance between the different investigated groups using SPSS version 15 (Chicago, IL, USA). Results were presented as means and standard deviations (S.D.). P values ≤0.05 were considered statistically significant.

RESULTS

Dual x-ray absorptiometric analyses revealed that rats in the glucocorticoid-treated group had lower bone mineral density and bone mineral content than those in the vehicle-treated group after 5 weeks of glucocorticoid treatment (Tables 3 and 4). Moreover, in Tables 1 and 2 glucocorticoid treatment significantly increased alkaline phosphatase activity, decreased blood osteocalcin level, and decreased bone matrix osteoprotegerin expression together with increase in expression of osteoclast-promoting factor RANKL (Figs. 1 and 2). Image analysis examination of bone tissue from glucocorticoid-treated rats confirmed trabecular bone loss in association with enlarged fat cell accumulation in the bone marrow compartment (Table 5).
The present results revealed that treatment with rimonabant significantly attenuated the inhibitory effects of glucocorticoid on bone mineral density, bone mineral content in skeletally immature rats (young group). On the contrary it deteriorated the BMD and BMC in the old group (Tables 3 and 4). There was a significant improvement in mean CBT and mean TBD in young group after rimonabant administration either alone or in combination with glucocorticoid (Table 5 data supported the dual X-ray analysis).

On the other hand rimonabant administration alone or with glucocorticoid in the old group produced a significant decrease in CBT and TBD.

The group of young rats receiving CB1 antagonist alone showed higher bone mass than those in the corresponding control group, however, there was a marked reduction in BMD and BMC in older age than those in the corresponding control group (Table 3). Rimonabant increased the expression of osteoprotegerin together with increase in activity of osteocalcin and inhibited alkaline phosphatase activity in comparison to glucocorticoid treated subgroup in the young rats (Table 2). On the contrary, in

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**Table 4. Effect of CB1 blocker and glucocorticoid on BMD and BMC of young rat tibial bone by DEXA.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>BMD (g/cm²)</th>
<th>BMC (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC2</td>
<td>0.092±0.021</td>
<td>0.211±0.052</td>
</tr>
<tr>
<td>MP2</td>
<td>0.076±0.018</td>
<td>0.106±0.039</td>
</tr>
<tr>
<td>RIM2</td>
<td>0.107±0.031</td>
<td>0.232±0.067</td>
</tr>
<tr>
<td>MP + RIM2</td>
<td>0.133±0.017</td>
<td>0.344±0.072</td>
</tr>
<tr>
<td>P value</td>
<td>0.002</td>
<td>0.001</td>
</tr>
</tbody>
</table>

All results are expressed as mean ± standard deviation (S.D.). Non significant: at P>0.05. Significant: at P<0.05. 2: skeletally immature rats (8 rats/group): NC2 (negative control); MP2 (methyl prednisolone group); RIM2 (rimonabant group); MP + RIM2 (rimonabant + methyl prednisolone group).

P value = ANOVA P value for comparison among old age groups; * = significance between control group and other groups; # = significance between methyl prednisolone group and rimonabant group or rimonabant + methyl prednisolone group; ∝ = significance between rimonabant group and rimonabant + methyl prednisolone group.

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Fig. 1. Expression of OPG in rat bone. Values were normalized to expression of S18 RNA levels and expressed in old relative to data from young rats. Values are means ± S.D. (8 rats/group). NC Group (negative control group), MP Group (methyl/prednisolone group), RIM Group (rimonabant group), MP + RIM Group (rimonabant + methyl/prednisolone group). 3 = means extremely significant statistically. * = significance between control group and other groups either for young or old age groups; # = significance between MP group and RIM group or MP + RIM group either for young or old age groups; ∝ = significance between RIM group and MP + RIM group either for young or old age groups.

Fig. 2. Expression of RANKL in rat bone. Values were normalized to expression of S18 RNA levels and expressed in old relative to data from young rats. Values are means ± S.D. (8 rats/group). NC Group (negative control group), MP Group (methyl/prednisolone group), RIM Group (rimonabant group), MP + RIM Group (rimonabant + methyl/prednisolone group). 3 = means extremely significant statistically. * = significance between control group and other groups either for young or old age groups; # = significance between MP group and RIM group or MP + RIM group either for young or old age groups; ∝ = significance between RIM group and MP + RIM group either for young or old age groups.
Table 5. Effect of CB1 blocker and glucocorticoid on CBT and TBD of rat tibial bone by image analysis.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Young CBT (µm)</th>
<th>Old CBT (µm)</th>
<th>P2 TBD (%) Young</th>
<th>Old TBD (%)</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>72.7±6.67</td>
<td>63.3±4.21</td>
<td>0.028</td>
<td>81.8±3.17</td>
<td>75.75±4.49</td>
</tr>
<tr>
<td>MP</td>
<td>56.2±5.46</td>
<td>49.2±5.05</td>
<td>0.09</td>
<td>65.86±3.33</td>
<td>57.4±2.15</td>
</tr>
<tr>
<td>RIM</td>
<td>71.6±1.73</td>
<td>50.4±7.7</td>
<td>0.001</td>
<td>79.4±2.47</td>
<td>59.09±5.3</td>
</tr>
<tr>
<td>MP + RIM</td>
<td>70.98±2.16</td>
<td>48.95±3.5</td>
<td>0.001</td>
<td>79.2±3.78</td>
<td>51.8±3.14</td>
</tr>
<tr>
<td>P1</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean cortical bone thickness (CBT) (µm) and mean trabecular bone density (TBD) (%) were obtained by measuring 5 fields/slide from 5 slides for each rat. The reading of each animal was considered as one variable. All results are expressed as mean ± standard deviation (S.D.). Non significant: P>0.05. Significant: P<0.05. NC (negative control); MP (methyl prednisolone group); RIM (rimonabant group); MP + RIM (rimonabant + methyl prednisolone group).

P1 = ANOVA P value for inter-comparison groups; P2 = ANOVA P value for comparison between young and old groups concerning CBT; P3 = ANOVA P value for comparison between young and old groups concerning TBD. * = significance between control group and other groups (young or old); # = significance between methyl prednisolone group and rimonabant group or rimonabant + methyl prednisolone group either for young or old.

Table 1 rimonabant in the old group showed more deterioration in bone remodeling markers in comparison to the osteoporosis subgroup. These parameters revealed more osteoporosis with combined rimonabant and glucocorticoid than glucocorticoid.
alone. Rimonabant alone in old rats (subgroup RIM1) showed osteoporotic indices like increase in levels of RANKL/OPG ratio, decrease in osteocalcin activity, together with increase in alkaline phosphatase activity.

**DISCUSSION**

Osteoporosis is a metabolic bone disease that is characterized by reduced bone mass and deterioration of bone microarchitecture (30, 31). By examining the present DEXA and morphometry bone results, they showed that a chronic glucocorticoid therapy was associated with low BMD and BMC with suggestion of low bone turnover and the high susceptibility of fractures, this finding agreed with other researches such as Sosa et al. (32) and Migliaccio et al. (33) who found that prednisolone administration induces apoptosis of both osteoblasts and osteocytes that lead to the suppression of bone formation and low BMD. Moreover, Surve et al. (34, 35) noticed that glucocorticoid decreases the periosteal mineralizing surface.

Bone resorption and formation are tightly orchestrated via the RANK/ RANKL/OPG system (36, 37). OPG is produced by osteoblast and plays a key role in the physiological regulation of osteoclastic bone resorption. It has no direct signaling capacity and acts by binding to its natural ligand which is known as RANKL. Ko et al. (38) found that this binding prevents activation of RANK receptor which is essential for osteoclast differentiation, activation and survival then inhibiting bone resorption. Furthermore, the inhibitory effect of OPG on bone resorption can also be explained as a modulator of RANKL half-life (39). The present results proved that supraphysiologic levels of glucocorticoid lead to an imbalance in the RANKL/OPG) ratio, with increasing serum and gene expression of RANKL and decreasing serum and gene expression of OPG. These parameters revealed osteoclastogenesis and accelerated bone resorption leading to a reduction in the skeletal mass which is the hallmark of the osteoporosis. This result agreed with that of McLaughlin et al. (40) who reported that glucocorticoids promote osteoclastogenesis via increasing RANKL and decreasing the OPG expression Also, the present study showed age-related OPG elevation as a compensatory phenomenon to slow down enhanced bone resorption.

This study proved the age-related reduction in bone mineralization together with reducing expression of OPG gene and increasing the expression of RANKL gene. However, serum calcium concentration did not show age-related reductions, this was different from humans in that rats were fed a nutritionally complete diet throughout aging. In addition, Elshal et al. (41) described that serum calcium concentration changes only in a critical situation, such as undernutrition or hyperparathyroidism.

Serum OC and ALP bone markers were lower with aging in this study. OC is a biomarker of bone formation and ALP is proportional to bone remodeling rates (42). These results suggested that the bone remodeling slows with growing in age in this rat model. Also, results revealed increasing susceptibility of osteoporosis and can be explained by Gimble et al. (43) who found that bone marrow stromal cells (MSCs) from elderly subjects have a reduced capacity to differentiate into osteoblasts and an increased capacity to differentiate into adipocytes, which leads to progressive accumulation of fat in the bone marrow space with increasing age.

Idris et al. (8) were first to report that genetic inactivation of the CB1 receptor results in high peak bone mass in young mice

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**Fig. 7.** The photomicrograph of a section in the tibia of old age rats received methyl prednisolone, showing a significant trabecular bone loss in association with enlarged fat cell accumulation in the bone marrow compartment. Hx. & E.; ×100.

**Fig. 8.** The photomicrograph of a section in the tibia of control normal old age rats showing mean cortical bone thickness and mean trabecular bone density. Hx. & E.; ×100.

**Fig. 9.** The photomicrograph of a section in the tibia of control normal young age rats showing mean cortical bone thickness and mean trabecular bone density. Hx. & E.; ×100.
with less osteoclasts and reduced bone resorption. These findings have led to the realization that cannabinoid receptors may play a significant role in the regulation of peak bone mass. The present study agreed with Idris et al. study (8) in increasing BMD and BMC in the skeletally immature rats treated with rimonabant, but our results went on to show marked osteoporosis with CB1 blocker with increasing age due to a defect in bone formation and accumulation of marrow fat. Idris et al. (15) observed that there was a significant increase in levels of CB1 transcript between 3 and 12 months of age in freshly isolated bone marrow cells from wild-type mice, demonstrating that CB1 expression is upregulated with age in the bone marrow compartment. They also observed direct effects of CB1 on osteoblast and osteoclast differentiation in vitro. This can provide our results a possible explanation for the age-related effects of CB1 antagonist of bone loss.

Tam et al. (11) speculated that CB1 stimulates bone formation by inhibiting release of catecholamines from peripheral nerves, the present study indicates that administration of CB1 blocker caused an increase in serum osteocalcin and decrease in serum ALP activities.

The present data revealed a reduction in RANKL gene expression and an increase in 86gene expression of OPG in the skeletally immature rats. This coincides with Idris et al. (15) who suggested that the defect in osteoclast formation in CB1 deficient mice is caused by a reduction in the sensitivity of osteoclast precursors to RANKL and a reduction in the ability of CB1 deficient osteoblasts to support osteoclast formation due to reduced RANKL expression.

Many studies explained the glucocorticoid effects on BMD and BMC in the old and young (35, 38), and also, the improvement in CBT and TBD in young rats after a CB1 blocker administration either alone or in combination with glucocorticoid, while in the old group, there was a significant decrease in CBT and TBD (13, 15, 17).

This work has important clinical implications in raising the possibility that cannabinoid receptor ligands may be of value therapeutically in enhancing peak bone mass and in preventing age-related osteoporosis, but indicates that antagonists may exert contrasting effects on the skeleton at different stages in life.

Conclusion

It has been concluded that CB1 receptor play age related different roles in bone turnover processes. So, the beneficial effects of CB1 blocker as rimonabant in modulating the majority of bone markers and improving bone mineral density and bone mineral content in order to preserve bone tissue against secondary osteoporosis induced by glucocorticoid therapy in young. It was suggested that CB1 receptor antagonist aggravates and may mediate age related osteoporosis, so, it should be prohibited in old age.

Abbreviations: CB1, cannabinoid receptor 1; BMD, bone mineral density; BMC, bone mineral content; OPG osteoprotegrin; RANKL, receptor activator of nuclear factor kappa B ligand; CBT, mean cortical bone thickness; TBD, mean trabecular bone density;

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Conflict of interest: None declared.

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