Efek Pemberian *Rat Recombinant Leptin* secara Intrahematoma terhadap Rasio Kalus, Volume Kalus dan Jumlah Sel Osteoblas pada Tikus Putih dengan Fraktur Femur

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ABSTRAK

Pendahuluan. Trauma cedera otak akan meningkatkan proses penyembuhan fraktur. Beberapa eksperimen dan studi observasional menunjukkan bahwa fenomena ini berkorelasi dengan leptin. Salah satu efek adalah perannya dalam proses metabolisme tulang. Leptin merangsang osteoblas untuk membuat lebih cepat proliferasi melalui produksi beta IGF-1 dan TGF. Di bidang penyembuhan patah tulang, leptin masih belum ditentukan peranannya, baik secara langsung maupun tidak langsung. Beberapa studi yang menghubungkan leptin untuk percepatan penyembuhan fraktur proses. Mana hal ini diamati pada pasien dengan patah tulang yang disertai dengan cedera otak traumatis. Hasilnya adalah diperoleh hubungan positif antara kadar leptin dengan proses penyembuhan fraktur. Tujuan dari penelitian adalah untuk mengetahui pengaruh injeksi leptin intrahematoma dalam proses pembentukan kalus (rasio kalus dan volume) dan jumlah sel osteoblas pada tikus putih (*Sprague Dawley Rat*) dengan fraktur femur.

Bahan dan cara kerja. Penelitian eksperimental dari 40 tikus *Sprague Dawley* dengan fraktur tulang femur kanan dan kemudian difiksasi dengan menggunakan intramedulla. Hewan ini kemudian dibagi menjadi 2 kelompok (20 tikus setiap kelompok) di mana kontrol kelompok tidak mendapatkan perlakuan lebih lanjut, sedangkan kelompok perlakuan mendapatkan perlakuan tambahan dengan injeksi *Rat Recombinant Leptin* secara intrahematoma sebanyak 100 ng. Pada hari 28, dilakukan evaluasi ulang secara radiologis untuk menentukan kalus dan volume rasio kalus. Lalu binatang diterminasi dan dilakukan pengambilan sampel tulang femur. Sampel ini kemudian diperiksa secara histologis untuk menentukan jumlah sel osteoblas yang terkandung pada kalus.

Hasil. Rasio kalus dan volume kalus didapatkan lebih besar pada kelompok perlakuan dibandingkan dengan kelompok kontrol ($p < 0,01$). Jika dilihat dari jumlah osteoblas, ditemukan bahwa jumlah sel osteoblas pada kelompok perlakuan lebih banyak dibandingkan kelompok kontrol ($p < 0,05$).

Simpulan. Pemberian tikus intrahematoma rekombinan leptin meningkatkan kuantitas kalus dan sel osteoblas dalam proses penyembuhan fraktur tulang femur pada tikus *Sprague Dawley Rat*.

Kata kunci: leptin, penyembuhan fraktur, rasio kalus, volume kalus, sel osteoblas

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Effects of Intrahematoma Rat Recombinant Leptin Injection on Callus Ratio, Callus Volume, and Osteoblast Cell Count in White Rat With Femoral Fracture

ABSTRACT

Introduction. Traumatic brain injury will enhance fracture healing process. Several experimental and observational studies show that this phenomena is correlated with leptin. Leptin stimulates osteoblasts to make more rapid proliferation through the production of IGF-1 and TGF beta. In the field of fracture healing, leptin is still undetermined role, either directly or indirectly. Several studies linking leptin to the acceleration of fracture healing process. Where this is observed in patients with fractures are accompanied by a traumatic brain injury. The result is obtained a positive relationship between leptin levels with fracture healing process. The objective of the study is to determine the effect of intrahematoma leptin injection in the process of callus formation (the callus ratio and callus volume) and osteoblasts count in white rats (Sprague Dawley Rat) with femoral fracture.

Materials and methods. An experimental study of 40 Sprague Dawley rats with right femoral bone fracture and then fixed using intramedullary wire. Animal is then divided into 2 groups (20 rats each group) where the control group did not get further treatment, while the treatment group get additional treatment of intrahematoma recombinant rat leptin injection as much as 100 ng. On day 28, we performed radiologic reevaluation to determine callus ratio and callus volume. The animals were then terminated and we took their sample of right femur bone. These samples were then examined histologically to determine osteoblast cells count contained in the callus.

Results. Callus ratio and callus volume were greater in the treated group compared with the control group ($p<0.01$). If seen from the number of osteoblasts, it was found that the number of osteoblast cells in the treated group more than the control group ($p<0.05$).

Conclusions. Administration of intrahematoma recombinant rat leptin increasing the quantity of callus and osteoblasts cells in the healing process of femoral bone fracture in Sprague Dawley rats.

Keywords: leptin, fracture healing, callus ratio, callus volume, osteoblast count

Introduction

The relationship between brain injury and fracture healing process has been widely noted in the literature, where patients with brain injury have the time in a more rapid fracture healing.[1,2] It is possible, because it was believed the substance or substances produced by the body after the brain trauma, which then stimulates the occurrence of this material is more rapid fracture healing.[3] Based on previous research conducted, it was found that in people with brain injury, produced a substance which is then circulated systemically through the blood which then influence the process of fracture healing.[3] This is supported by in vitro studies showing that severe brain injury patient serum can provide stimulation to the muscle cells to mesenchymal cells differentiate osteoblastic and speed up the process.[1,3]

Several humoral and hormonal factors are suspected to play a role in this process. One such factor is leptin, in which leptin is a hormone derived from fat cells, which have been known to go play a role in bone metabolism.[4] In the study conducted by Wang (2011), found that leptin levels increased in the brain injury group compared to controls, especially in the second week ($p <0.05$), fourth ($p <0.01$) and eighth ($p <0.05$).[5] In the same study, Wang et al (2011) states that the existence of the close contact between elevated levels of leptin and the degree of callus formation in fracture healing process.[5] So researchers are interested in knowing the effect of leptin is the process of fracture healing. Until now, we have not yet known about the effects of leptin administration directly to the process of fracture healing.

Materials and methods

This study is experimental study design. Experimental animals were taken from *Sprague Dawley Rat* population through randomization of the right femur closed diaphyseal fracture. This randomization enables all experimental animals received the same possibility to be included as the treatment groups. Preparing for the research that experimental animals amounted to 40 *Sprague Dawley Rat* (20 each group). Procedures each study group (experimental and control group) following closed fracturization[6], insertion of intramedullary wire and injection of rat recombinant leptin intrahematoma (100 ng) with aseptic procedures and use of sterile equipment in each rat.

Each sample was undergone internal fixation using K-wire 1.2 mm diameter with retrograde technique through parapatellar approach. Then we did closed fracturization technique of right femur and closed the incision wound with stitches. We evaluated with plain x-ray. (Figure 1) In experimental group, we gave 100 ng single injection of rat recombinant leptin intrahematoma shortly after fracturization, but no further procedure in control group. Both group is maintenance with the same environment and nutrition. Data was obtained on day 28, using plain x-ray and callus histological examination. We calculated the callus ratio and volume by measuring callus diameter, femur diameter, and length of callus; also determined osteoblast cell count using histological examination (semiquantitative).

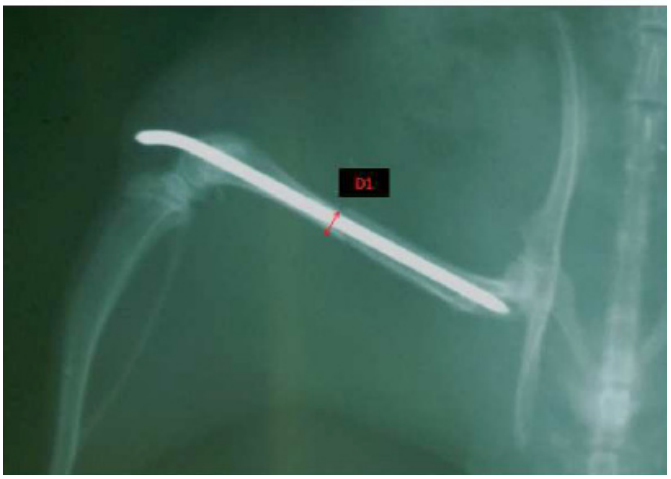


Figure 1. Plain X-ray of right femur after retrograde intramedullary wiring and closed fracturization; also evaluation of femur diameter (red arrow)

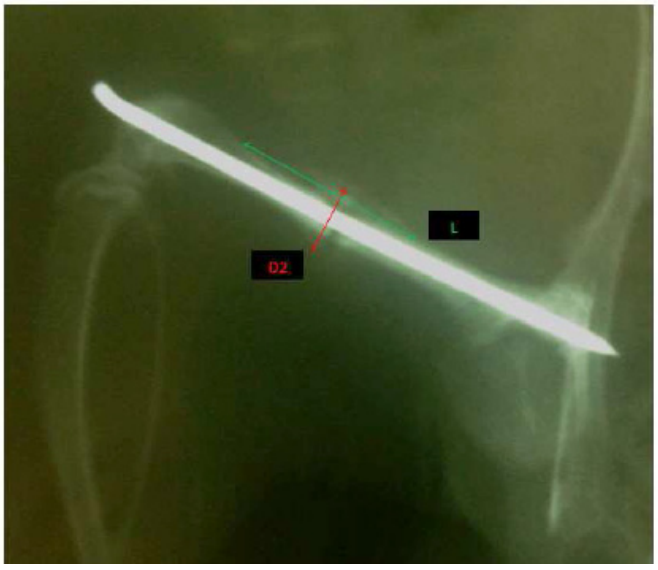


Figure 2. Plain X-ray right femur on day 28 after manipulation; also evaluation of callus diameter (red arrow) and length of callus (green arrow)

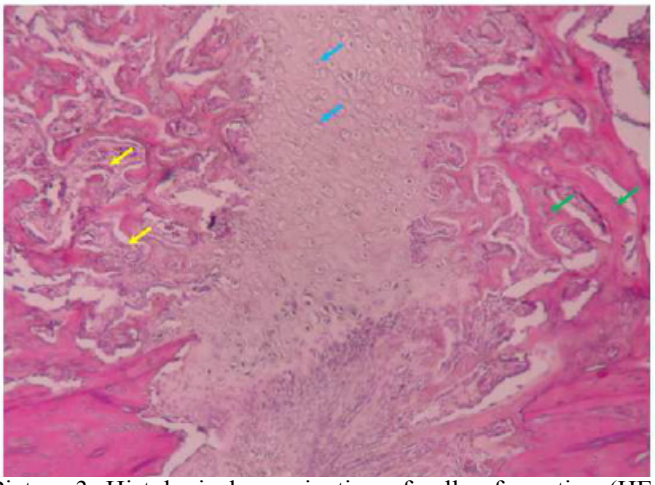
Results

From the calculation was done, we found that callus ratio (day 28) in experimental group was greater than control group (1.93±0.26 v.s 1.36±0.16, $p<0.01$). Figure 2 shows radiograph taken at day 28. Meanwhile, if seen from the results of calculating the volume of callus, it was found that the callus volume (day 28) in experimental group higher than the control (112.25±42,9 v.s 42.38±7.1, $p<0.01$)). The results of semiquantitative calculation of the number of osteoblast cells (on day 28) showed that the number of osteoblast cells in the experimental group higher than control group (2.18±0.73 v.s 1.53±0.77, $p<0.05$). FIgure 3 shows histological finding on at day 28.

Discussions

The relationship of the ratio of callus leptin levels have been studied and have a positive correlation.[5] Based on existing literature, leptin also has a positive effect on the process of formation or bone metabolism. The effect is a peripheral effect of leptin where leptin affects the production of osteoprotegerin (OPG) and Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), as well as Neuropeptide Y (NPY) and Hypothalamic Osteoblast Inhibitory Factor (HOBIF). Increased production of OPG and RANKL inhibition of production, would lead to inhibition of osteoclast cell activity. Leptin is also a positive direct effect on osteoblast activity.[7] Leptin also causes osteoblastic cells to make IGF-I and TGF-betas, which in turn stimulate the proliferation of osteoprogenitor cells (osteoprogenitor), stimulate bone matrix mineralization, and prevent osteoblasts and osteocytes from committing apoptotic suicide.[7]

The results obtained in this study is the occurrence



Picture 3. Histological examination of callus formation (HE staining); shows osteoblast cells (yellow arrow), chondrocyte cells (blue arrow), and osteocyte cells (green arrow)

of differences in the ratio of callus and callus volume between treatment groups with control groups. The ratio of callus in the experimental group (day 28) is significantly higher than control group. This is consistent with research that found previously, where the ratio of the fracture callus group of patients with brain injury accompanied by 1.9, while patients with a fracture without brain injury is equal to 1.3 or there is a difference of 46% in both groups (range 37 - 50%, $p < 0.01$).[3]

Also, the callus volume in experimental group is significantly higher than control group. It is also similar to studies conducted by Wang L. et al (2011), in which the acquired volume of callus in the treated group at 122.74 ± 9.65 mm 3 (at week 4), whereas in the control group of 70.23 ± 7.19 mm 3 (at week 4) with $p < 0.05$.[5] When comparing osteoblast cell count, the experimental group is significantly higher than control group. It is also shown from a similarstudy, in which there are significant differences on the ability of the callus cell proliferation ($p < 0.05$).[5] In another study also found that leptin stimulates osteoblastic cell proliferation in humans, de novo collagen synthesis, and mineralization processes.[8] Leptin encourage the growth of osteoblasts and chondrocytes in culture medium.[9] Anabolic effect is also consistent with the data of Thomas et al. (1999) in human bone marrow stromal cells suggests that leptin causes differentiation into osteoblast phenotype and that leptin increases the synthesis of bone matrix proteins such as collagen type I and osteocalcin.[10]

Conclusions

Intrahematomal rat recombinant leptin injection significantly induced callus formation and also increasing osteoblast cell in fracture healing process.

References

1. Boes M, Kain M, Kakar S, Nicholls F, Cullinane D, Gerstenfeld L, et al. Osteogenic effects of traumatic brain injury on experimental fracture- healing. J Bone Joint Surg Am. 2006;88:738-43.
2. Miller MD. 2008. Basic Sciences; In: Review of Orthopaedics, 5th ed. Saunders Elsevier.pp 11-15.
3. Cadosch D, Toffoli AM, Gautschi OP, Frey SP, Zellweger R, Skirving AP, et al. Serum after traumatic brain injury increases proliferation and supports expression of osteoblast markers in muscle cells. J Bone Joint Surg Am. 2010;92:645-53.
4. Wei Y, Wang L, Clark JC, Dass CR, Choong PF. Elevated leptin expression in a rat model of fracture and traumatic brain injury. J Pharmacy Pharmacol.2008;60:1667–72.
5. Wang L, Yuan JS, Zhang HX, Ding H, Tang XG & Wei YZ. Effect of leptin on bone metabolism in rat model of traumatic brain injury and femoral fracture. Chin J Traumatol. 2011;14(1):7-13.
6. Bonnarens F, Einhorn TA. Production of a standard closed fracture in laboratory animal bone. J Orthop Res.1984(2):97-101.
7. Whitfield JF. Leptin -- A New Member of the Bone Builders' Club?, MedGenMed.2002(4); no.3. Available at: http://www.medscape.com/viewarticle/439243.
8. Gordeladze JO, Drevon CA, Swersen U,Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. J Cell Biochem.2002;85(4):825-36.
9. Cornish J, Callon KE, Bava U, Lin C, Naot D, Hill BL, et al. Leptin directly regulates bone cell function *in vitro* and reduces bone fragility *in vivo*.J Endocrinol. 2002;175:405–15.
10. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B & Riggs BL. Leptin acts on human marrow stromalcells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinol.1999;140:1630-8.