Original Article

Effectiveness of Strontium Ranelate in the Treatment of Rat Model of Legg–Calve–Perthes Disease

Abstract

Background: Legg–Calve–Perthes disease (LCPD) causes osteonecrosis of the femoral head (ONFH) by temporarily interrupting the blood supply in children. Even with potential toward bone regeneration and revascularization in LCPD, the prognosis depends on the deformity of femoral heads, and successful rate with the current treatments varies. Antiresorptive therapy such as bisphosphonate, which maintains mechanical stability of the femoral head by inhibiting necrotic bone resorption, has proven effective in animal models. However, concerns on simultaneous decline in bone turnover rate still leave room for improvement. Strontium ranelate with dual effect on inhibiting bone resorption and accelerating bone formation is presumed to be an ideal therapy for reserving sphericity of femoral heads in LCPD. **Materials and Methods:** In this study of a rat model of ONFH, randomized groups of rats treated with strontium ranelate or normal saline are compared at different time points in analysis of radiological, histological, and bone morphometric changes. Gait analysis was also compared between the two groups. **Results:** The group treated with strontium ranelate recovered their normal gait earlier than the control group did. Bone density, trabecular thickness, sphericity of the femoral head, and bone regeneration potential were also preserved in the strontium ranelate group. **Conclusion:** Strontium ranelate effectively prevented collapse of the ischemic femoral head and enhanced trabecular thickness in the rat model of LCPD. Hopefully, this preclinical experiment can improve the effectiveness of strontium ranelate treatment for pediatric ONFH.

Keywords: *Legg–Calve–Perthes disease, osteonecrosis, strontium ranelate*

MeSH terms: *Strontium, Perthes disease, osteonecrosis*

Yu-Pin Chen, Amyla Tan[2], Wei-Pin Ho, Tai-Yuan Chuang, Wei-Chuan Chen[1], Chih-Hwa Chen[2,3]

Department of Orthopaedic surgery, Wan Fang Hospital, School of Medicine, College of Medicine, Taipei Medical University, [2]Department of Orthopaedic Surgery, Taipei Medical University Hospital, School of Medicine, College of Medicine, Taipei Medical University, [3]Bone and Joint Research Center, Department of Orthopedics and Traumatology, Taipei Medical University Hospital, School of Medicine, College of Medicine, Graduate Institute of Biomedical Materials and Tissue Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, [1]Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Taoyuan, Taiwan

Address for correspondence:
Prof. Chih-Hwa Chen, Department of Orthopaedic Surgery, School of Medicine, College of Medicine, Taipei Medical University Hospital, Taipei Medical University, Taipei 11031, Taiwan.
E-mail: afachen@doctor.com

Access this article online

Website: www.ijoonline.com

DOI: 10.4103/ortho.IJOrtho_437_16

Quick Response Code:

Introduction

Legg–Calve–Perthes disease (LCPD), a disease of unknown etiology, causes osteonecrosis of the femoral head (ONFH) by temporarily interrupting the blood supply in children.[1,2] Unlike adult-type ONFH, osteonecrosis in childhood has potential for bone regeneration and revascularization of the femoral head. However, the long term outcome still depends on the shape of the femoral head, age at onset, and the extent of the femoral head involvement.[1,3,4] The treatment goal is to prevent osteoarthritis later in adult life by minimizing femoral head deformity during the fragmentation stage of the disease. The current treatment strategy for LCPD is restricting weight-bearing activity and increasing hip joint containment, but success rates vary.[3,5] Therefore, improved treatments for LCPD are still needed.

In terms of pathophysiology, osteoclasts eliminate the necrotic area secondary to bone infarction during the fragmentation stage of LCPD, which decreases the ability of the femoral head to withstand pressure.[2] Collapse of ischemic femoral head ensues. Bisphosphonates, which interfere with bone resorption by inhibiting osteoclast function, have proven effective for preserving femoral head sphericity in animal models of LCPD.[6-11] However, the drawbacks of bisphosphonates treatment are reduced bone turnover rate and delayed bone regeneration.[9]

Strontium ranelate, an anabolic agent with proven anti-fracture activity, has proven effective for treating postmenopausal osteoporosis.[12] Strontium ranelate improves bone formation and resorption by activating calcium receptors, localized on osteoblasts and osteoclasts, and the influence on the OPG/RANKL system.[13-15] Therefore, we hypothesized that strontium ranelate can also prevent collapse of the femoral head in treating LCPD. Our hypothesis was tested

How to cite this article: Chen YP, Tan A, Ho WP, Chuang TY, Chen WC, Chen CH. Effectiveness of strontium ranelate in the treatment of rat model of legg–calve–perthes disease. Indian J Orthop 2018;52:380-6.

in a young female Wistar rat model of traumatic ONFH to assess the effect of intervention with strontium ranelate in the treatment of LCPD.

Materials and Methods

Animal model design and treatment protocol

All procedures were performed according to the Guide for the Care and Use of Laboratory Animals and approved by the Committee of Experimental Animal Sciences of Taipei Medical University. An LCPD model was established according to the previous inductive protocols.[16] Twelve female 12-week-old Wistar rats (average weight, 283 g) were anesthetized by intramuscular injection of a mixture of 50 mg/kg ketamine and 10 mg/kg xylazine. After preparation of the right lower extremity in aseptic manner, an incision was made over the right hip, and we manually dislocated the femoral head from the acetabulum and cut down the ligamentum teres.[16] The periosteum at the base of the femoral neck with reflected fibers of the capsule was circumferentially divided from the bone. After relocation of the femoral head, the joint capsule and gluteal muscles were repaired in sequence, and then the skin was closed in layers. The rats were housed in spacious cages so that perambulation would be unhindered. During the experiment, all rats were freely accessible to water and routine feeding.

By the 2nd week after surgery, sclerotic changes in the operated femoral head, which were clinically similar to those in the ischemic stage of LCPD (the Waldenström classification),[17] were confirmed in all rats with an X-ray machine (Hewlett Packard, McMinnville, USA) under anesthesia by an orthopedist. The 12 rats were then randomly divided into two groups. The control group was Group I (n = 6) which received normal saline orally each day. The experimental group was Group II (n = 6) which was given 500 mg/kg strontium ranelate orally each day (Protos, Servier, France). The animals were bred in groups of four in cages with liberty of standing erect while feeding and access to food and water. The contralateral (left), uninvolved femoral heads of the animals in the control group were also used as normal controls to reduce unnecessary use of animals.

Footprint analysis before euthanization

All experimental animals also underwent footprint analysis on the 2nd, 4th, 6th, and 8th weeks after operation. After the hind feet were dipped in carbon ink, the rat was placed in a paper-lined track. We analyzed the footprints left on the paper as the rats moved along the walkway.

In injured rats, walking is generally antalgic, i.e., the injured leg has a shorter gait stance duration as compared to the noninjured leg.[18] The two footprint measurements were taken in this study: injured gait-stance distance (IGSD), defined as the distance between toe tips of two consecutive footprints in the stance phase of gait cycle of the operated leg, and uninjured gait-stance distance (UGSD), defined as the distance between toe tips of two consecutive footprints in the stance phase of the gait cycle of the nonoperated leg [Figure 1]. These parameters were adapted to the antalgic gait index (AGI) with the formula: AGI = (IGSD − UGSD)/UGSD × 100. Theoretically, AGI approaches zero if the injured leg recovers. Therefore, IGSD and UGSD should not have a large difference.

For each animal, AGI data were recorded for nine footprints in the middle of the 20 steps taken by rats walking in the alley. The AGI was expressed as mean ± standard deviation (SD) for comparison between groups.

Radiographic assessment of epiphyseal quotient after euthanization

All animals were euthanized in a CO_2 chamber 8 weeks after surgery. After euthanization, both femurs from each rat were obtained and radiographically analyzed by an X-ray machine (Hewlett Packard, McMinnville, USA). Deformity of the femoral head was quantified by the epiphyseal quotient, which is defined as the maximum height of the epiphysis divided by its maximum diameter.[19] As the femoral head flattens, height of the epiphysis decreases but diameter of epiphysis increases, so the epiphyseal quotient decreases. The epiphyseal quotient for the anteroposterior radiograph of each rat was measured by a blinded radiologist.

Micro-computed tomography and morphometric assessment

The femoral heads were fixed in periodate-lysine-paraformaldehyde (PLP) fixative and prepared for further

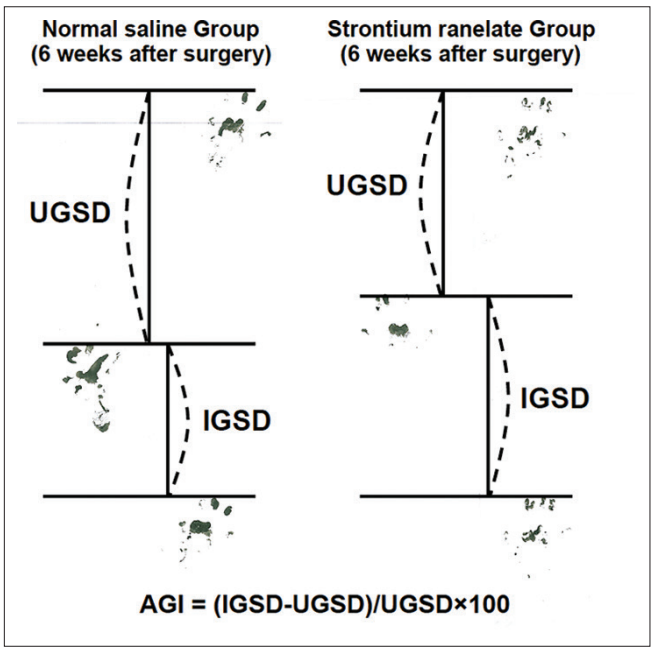

Figure 1: Footprints analysis for the antalgic gait index, shorter injured gait-stance distance than uninjured gait-stance distance in the normal saline group on the 6th weeks after surgery

scanning by micro-computed tomography (micro-CT). Bruker SkyScan 1176 (Kontich, Belgium) was used to scan samples at a resolution of 9 μm. Scanning was performed at 65 keV, 385 μA, 1050 ms of exposure time, and with a 0.5-mm aluminum filter. Sections were reconstructed with GPU-based scanner software (NRecon, Bruker, USA). The grayscale was based on Hounsfield unit and the validated calcium standards were scanned as its density reference. A CTAn (SkyScan, Bruker, USA) was used to analyze the area of epiphysis, which was identified by referring to the growth plate. Specific regions of interest (100 μm × 100 μm × 100 μm) were also randomly selected within each individual sample for further analysis. The three-dimensional (3D) morphometric indices for ratios of trabecular bone thickness, trabecular bone number, ratio of bone volume (BV) to total volume, and bone mineral density of target volume were then calculated by CTAn software. The CTVox (Version 3.0, SkyScan, Bruker, USA) software was used for 3D reconstruction.

Histological examination and assessment of bone regeneration

All femoral head samples were fixed in PLP, decalcified in an EDTA-G solution, and embedded in paraffin.[20] For histology study, the samples were cut into 5-μm thick section, mounted on slides, and stained with Masson's trichrome stain to identify areas of bone necrosis and new bone formation. The sections were also stained for both alkaline phosphatase and tartrate-resistant acid phosphatase analyses.[20] In these sections, we estimated osteoclast surface (Oc.S, %) calculated by percentage of multinucleated red surface among cells and osteoblast surface (Ob.S, %) measured by all cuboidal blue surface associated cell profiles. The investigator who performed the measurements was blinded to the treatment groups. A tissue area, which was at least 500 mm away from the surface of femoral head to ensure that the analysis excluded primary spongiosa, was sampled at least 40 mm^2 from each osseous epiphysis. All histological indices were estimated according to the guideline from American Society for Bone and Mineral Research.[21]

Statistical analysis

In the calculations of micro-CT morphometric and histomorphometric indices and in measurements of the epiphyseal quotient, independent *t*-tests revealed variations among the treatment groups (version 19.0, SPSS Inc., Chicago, IL, USA). Differences between the surgically treated groups and the normal femoral heads were compared by performing paired sample *t*-tests (significance: $P < 0.05$). The data were presented as means and SDs.

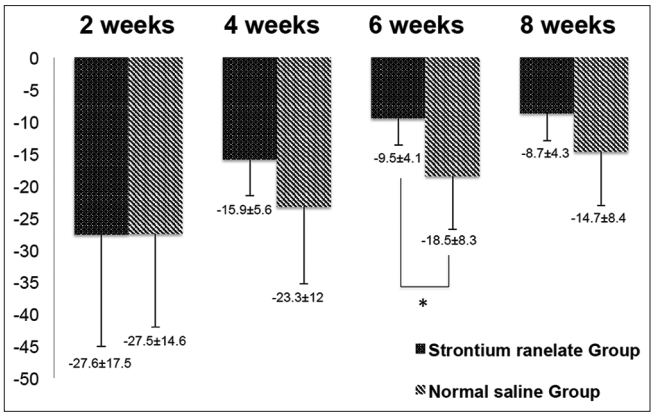

Figure 2: A bar diagram showing comparison of antalgic gait index between groups (*significant difference with $P < 0.05$)

Results

Radiographic evaluation and footprint analysis before euthanization

Footprint analysis results show that AGIs were similar between the strontium ranelate and the normal saline group on the 2nd week after the inductive operation for ONFH [Figure 2]. In the strontium ranelate group, however, AGI was significantly higher in the strontium ranelate group compared with the normal saline group ($P = 0.04$). Over time, however, improvement was greater than that in the normal saline group. This implied that the strontium ranelate treatment gradually improved the gait in rats with inductive ONFH.

Radiographic assessment of epiphyseal quotient after euthanization

Under gross image, the femoral head in the normal saline group showed significant deformity compared with that in the strontium ranelate group and normal control group [Figure 3A1-A3]. Radiographic assessment of the femoral heads after euthanization also demonstrated significant collapse with visible femoral neck resorption in the normal saline group when comparing with the other two groups [Figure 3B1-B3]. The normal saline group also had a lower mean epiphyseal quotient compared to the strontium ranelate group [Figure 4]. Instead, femoral heads under the treatment of strontium ranelate relatively reserved sphericity of normal femoral head. The mean epiphyseal quotient of the strontium ranelate group was significantly higher than that in the normal saline group ($P = 0.001$).

Micro-computed tomography and morphometric assessment

3D micro-CT assessment clearly showed the deformity of the epiphysis with cavitation in the normal saline group and preserved sphericity of the femoral heads in the strontium ranelate group [Figure 5]. Quantitative assessment of the micro-CT sections of each femoral head for trabecular

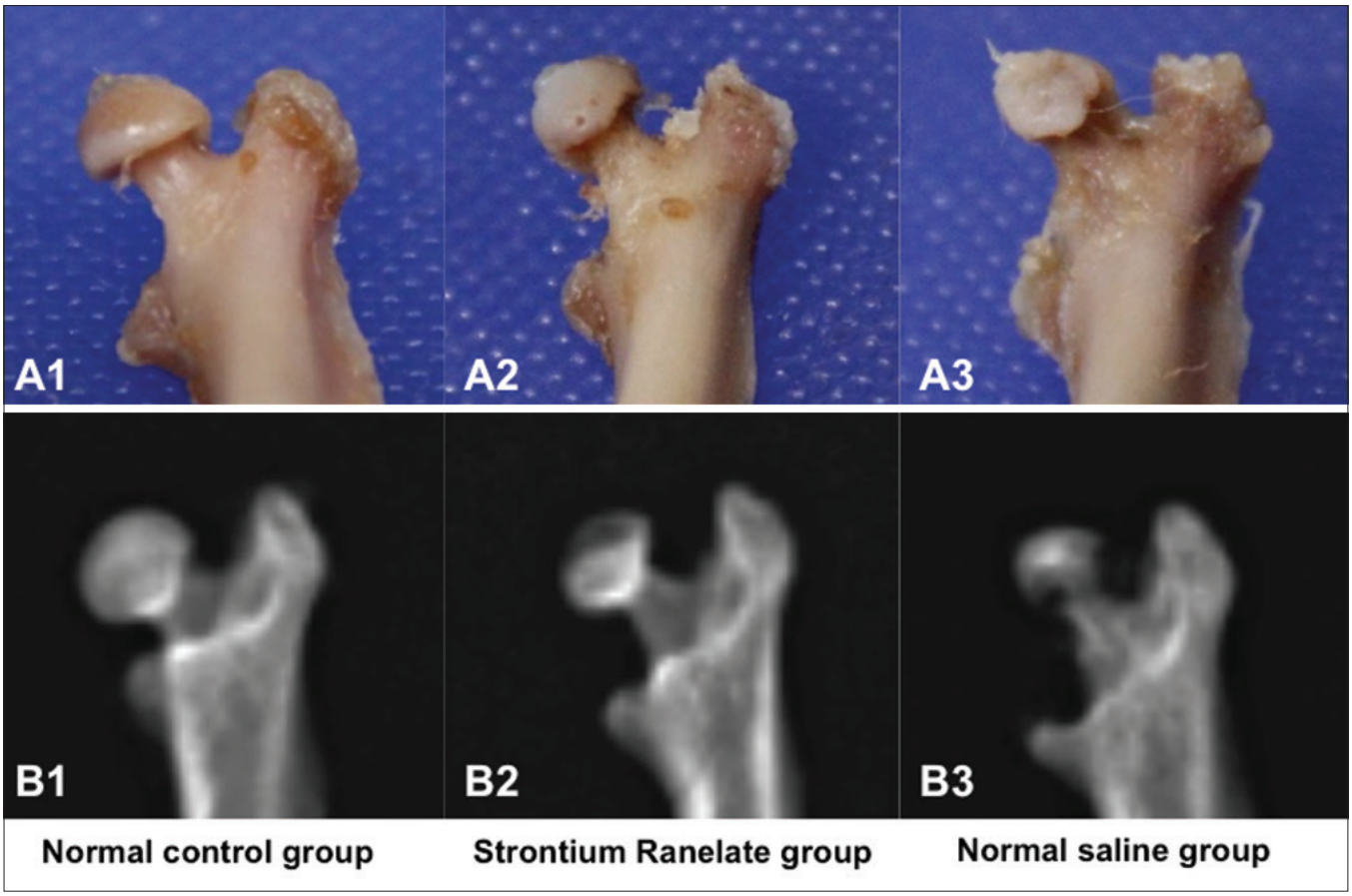

Figure 3: Morphological change after euthanization (A: Gross images of the femoral heads; B: Representative radiographs of the femoral heads)

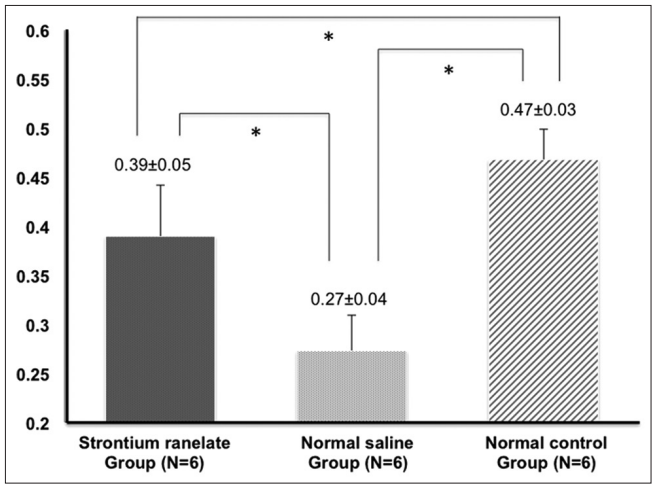

Figure 4: A bar diagram showing calculation of epiphyseal quotient (*significant difference with *P* < 0.05)

bone parameters [Table 1] revealed a significantly higher mean BV percent in the strontium ranelate group compared with the normal saline group (P = 0.04). The mean trabecular number (per millimeter) was significantly lower in the strontium ranelate group compared with the normal control group (P = 0.02) but did not significantly differ between the strontium ranelate group and the normal saline group (P = 0.31).

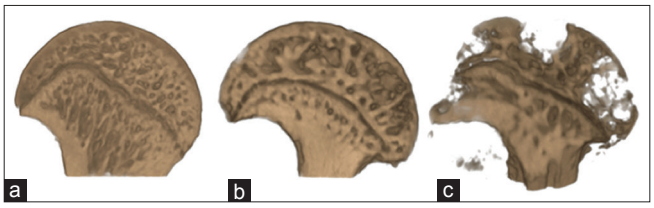

Figure 5: Three-dimensional micro-computed tomography assessment of the femoral heads (a) Normal control group; (b) Strontium ranelate group; (c) Normal saline group)

The mean trabecular thickness was significantly higher in the strontium ranelate group compared with the normal saline group (P = 0.04). Moreover, in the strontium ranelate groups, mean bone mineral density was significantly lower than that in the normal control group (P < 0.01) but significantly higher than that in the normal saline group (P = 0.04).

Histological examination and assessment of bone regeneration

The Masson's trichrome staining of the femoral head in the normal saline group showed notable deformity and collapse of epiphyseal height compared with that in the normal control group [Figure 6a and c]. Large areas of bone resorption with fibrovascular tissue invasion were also identified in the normal saline group [Figure 6c]. The

Table 1: Micro-computed tomography results

Parameters	Normal control group (*n*=6)	Strontium ranelate group (*n*=6)	Normal saline group (*n*=6)
BV/TV (%)	55.6±5.8	53.9±8.4	43.6±5.0[a,b]
Trabecular thickness (μm)	165±27	188±44	134±26[a]
Trabecular number (mm^{-2})	3.5±0.3	2.9±0.3[a]	3.4±0.8
Bone mineral density (g/cm^2)	0.114±0.003	0.105±0.002[a]	0.098±0.007[b]

[a]*P*<0.05 compared with normal control group, [b]*P*<0.05 compared with strontium ranelate group. TV=Total volume, BV=Bone volume

Table 2: Histomorphometry results for osteoclast and osteoblast surface

Parameters	Normal control group (*n*=6)	Strontium ranelate group (*n*=6)	Normal saline group (*n*=6)
Oc.S (%)	3.48±0.52	2.53±0.86[a]	3.81±0.85[b]
Ob.S (%)	12.33±3.68	11.83±3.31	16.41±3.56[b]

[a]*P*<0.05 compared with normal control group, [b]*P*<0.05 compared with strontium ranelate group. Oc.S=Osteoclast surface, Ob.S=Osteoblast surface

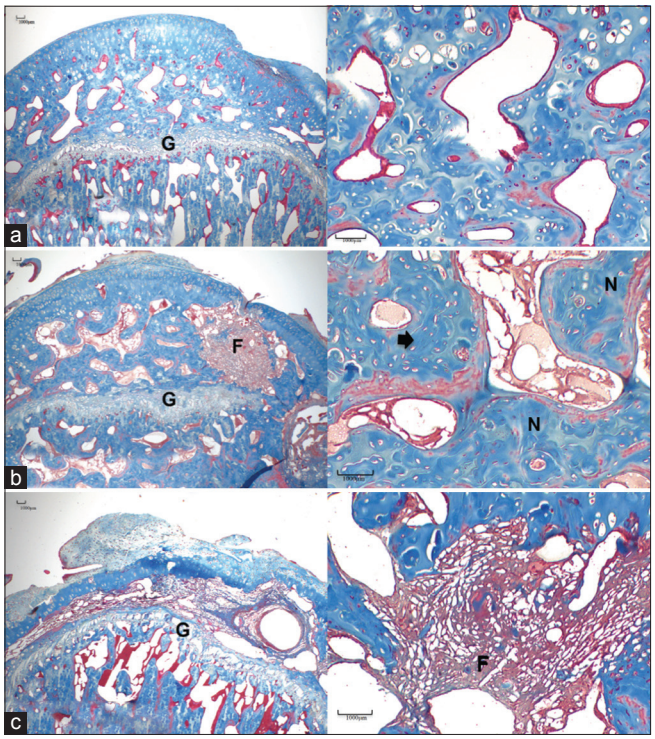

Figure 6: Histological assessment of the specimens (a) Normal control group; (b) Strontium ranelate group; (c) Normal saline group (area of live bone tissue [arrow]; G: Growth plate; F: Fibrovascular tissue; N: Necrotic bone debris with empty lacuna; scale bars 1000 μm)

strontium ranelate group exhibited higher sphericity of the femoral head compared with the normal control group and with the normal saline group [Figure 6b]. Moreover, except for area of bone debris with empty lacuna among the trabecular matrix, area of live bone tissue with mark osteocyte located in the lacuna was also found in the strontium ranelate group.

The Oc.S was significantly lower in the strontium ranelate group than the normal control and the normal saline groups (P = 0.04 and 0.03, respectively) [Table 2]. However, Oc.S did not significantly differ between the normal control and normal saline groups. The Ob.S was significantly lower in the strontium ranelate group compared to the normal saline group (P = 0.04) but was not significantly different between the strontium ranelate and the normal control groups.

Discussion

For most orthopedic surgeons, treating LCPD is a clinical challenge. Preventing femoral head deformity is the most important goal when treating LCPD. However, current surgical treatments have limited efficacy in achieving a normal hip at maturity.[1,5] Therefore, new treatments are needed that specifically address the biologic and mechanical aspects of the disease.

Vascular disruption is regarded as a key factor in the pathogenesis of LCPD.[2] Models of disrupting vascular supplement form the femoral head in growing animals is believed to mimic the models of LCPD. However, these models are more relevant to traumatic ONFH. In a study by Norman *et al*., the pathogenesis of LCPD was simulated by surgical induction of ONFH in a rate model by cutting the ligamentum teres and stripping the periosteum.[16] Histology of the bone marrow revealed necrosis in the 1st week after inductive surgery and remodeling of the femoral heads with new bone formation on the 5th week after surgery. Utilizing the rat model for LCPD in our studies revealed gross disruption of the femoral head architecture in the normal saline group but predominantly preserved under the treatment of strontium ranelate.

Several studies have reported that inhibiting osteoclast activity prevents necrotic bone resorption, which helps maintain mechanical stability of the femoral head.[6-11] Little *et al*. reported that zoledronic acid treatment improves retention of the femoral head structure after traumatic osteonecrosis in young Wistar rats.[10] Kim *et al*. also showed that inhibiting the interaction between RANK and RANKL with osteoprotegerin preserves the femoral head structure after ischemic osteonecrosis in a young piglet model.[8] In both studies, however, inhibiting osteoclast activity also significantly reduced the Ob.S, especially in comparison with the normal control group. The inevitable decrease in the bone turnover rate therefore delayed new bone formation.

Strontium ranelate has been used for simultaneously inhibiting osteoclast activity and promoting osteoblast function, rebalancing bone turnover in favor of bone formation.[22] Our study showed that strontium ranelate not only inhibited osteoclast function (by decreasing Oc.S) but also reserved sphericity of the affected femoral heads,

especially when compared with the normal saline group. Moreover, unlike the results obtained by antiresorptive agents in other studies,[8,10] strontium ranelate significantly enhanced trabecular thickness and maintained Ob.S when compared with the normal control group. Although lack of dynamic evaluation for mineralized surface and bone formation rate to directly demonstrate new bone formation in the strontium ranelate group in our study, our experimental results imply that strontium ranelate can potentially preserve femoral head architecture without significantly reducing bone turnover rate in the treatment of rats model of LCPD.

Several limitations of the proposed treatment for LCPD are noted. The two major drawbacks of the study are the lack of a dynamic evaluation for new bone formation and the lack of direct comparison between strontium ranelate and other antiresorptive agents. Second, the rat model of traumatic ONFH used may not accurately represent the progression of LCPD in children. Third, since the necrotic area of ONFH is avascular, the parenteral strontium ranelate may not reach the central necrotic portion of femoral heads. Moreover, nonweight-bearing treatments have proven to be mechanically protective and to decrease the femoral head deformity in animal models of LCPD[23] which was not restricted in our study. We believe that parenteral use of strontium ranelate and the lack of restriction on weight-bearing stress in our experimental design may also cause a negative influence on the treatment outcome.

Theoretically, except for inhibiting necrotic bone resorption, stimulating new bone formation is also important in treatment of LCPD to accelerate bone regeneration and maintain mechanical stability of femoral heads. Combined therapy with intraosseous injection of antiresorptive agents and bone morphogenetic protein-2 has proven more effective in preserving femoral head sphericity than single therapy with antiresorptive agents alone in immature pig models of LCPD.[24,25] Animal models of ONFH have also shown that stem cell treatment is effective for stimulating new bone formation in preventing femoral head collapse.[26,27] Our experimental results herein reveal a simple and effective therapy with strontium ranelate for reserving femoral head sphericity without significantly reducing bone turnover rate in treatment of the rats model of LCPD. Even with promising results in treatment of the animal model of LCPD, strontium ranelate has been reported to have undesirable side effects including allergy and increased risk for cardiovascular events in systemic use.[28] Locally intraosseous injection into the affected femoral head is our aim to avoid systemic side effects especially while applying strontium ranelate to pediatric groups. Considering bone regeneration effect on the human preadipocyte by strontium,[29] our laboratory will further study the effectiveness of intraosseous injection of strontium ranelate combined with stem cell therapy for new bone formation in the future studies.

Conclusion

Our experiment simply demonstrates that strontium ranelate can effectively prevent collapse of the ischemic femoral head and enhance trabecular thickness in the rat model of LCPD. Hopefully, this preclinical experiment can improve the effectiveness of strontium ranelate treatment for pediatric ONFH.

Financial support and sponsorship

The authors would like to thank Wan Fang Hospital (grant 103-wf-eva-15) for financially supporting this research.

Conflicts of interest

There are no conflicts of interest.

References

1. Kim HK. Legg-Calvé-Perthes disease. J Am Acad Orthop Surg 2010;18:676-86.
2. Kim HK. Pathophysiology and new strategies for the treatment of Legg-calvé-Perthes Disease. J Bone Joint Surg Am 2012;94:659-69.
3. Herring JA, Kim HT, Browne R. Legg-Calve-Perthes disease. Part II: Prospective multicenter study of the effect of treatment on outcome. J Bone Joint Surg Am 2004;86-A:2121-34.
4. Joseph B. Prognostic factors and outcome measures in Perthes disease. Orthop Clin North Am 2011;42:303-15, v-vi.
5. Wiig O, Terjesen T, Svenningsen S. Prognostic factors and outcome of treatment in Perthes' disease: A prospective study of 368 patients with five-year followup. J Bone Joint Surg Br 2008;90:1364-71.
6. Peled E, Bejar J, Barak M, Orion E, Norman D. Core decompression and alendronate treatment of the osteonecrotic rat femoral head: Computer-assisted analysis. Int J Exp Pathol 2013;94:212-6.
7. Kim HK, Sanders M, Athavale S, Bian H, Bauss F. Local bioavailability and distribution of systemically (parenterally) administered ibandronate in the infarcted femoral head. Bone 2006;39:205-12.
8. Kim HK, Morgan-Bagley S, Kostenuik P. RANKL inhibition: A novel strategy to decrease femoral head deformity after ischemic osteonecrosis. J Bone Miner Res 2006;21:1946-54.
9. Little DG, McDonald M, Sharpe IT, Peat R, Williams P, McEvoy T. Zoledronic acid improves femoral head sphericity in a rat model of perthes disease. J Orthop Res 2005;23:862-8.
10. Little DG, Peat RA, Mcevoy A, Williams PR, Smith EJ, Baldock PA. Zoledronic acid treatment results in retention of femoral head structure after traumatic osteonecrosis in young Wistar rats. J Bone Miner Res 2003;18:2016-22.
11. Kim HK, Su PH. Development of flattening and apparent fragmentation following ischemic necrosis of the capital femoral epiphysis in a piglet model. J Bone Joint Surg Am 2002;84-A: 1329-34.
12. Przedlacki J. Strontium ranelate in post-menopausal osteoporosis. Endokrynol Pol 2011;62:65-72.
13. Bonnelye E, Chabadel A, Saltel F, Jurdic P. Dual effect of strontium ranelate: Stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption *in vitro*. Bone 2008;42:129-38.
14. Canalis E, Hott M, Deloffre P, Tsouderos Y, Marie PJ. The divalent strontium salt S12911 enhances bone cell replication

and bone formation *in vitro*. Bone 1996;18:517-23.
15. Marie PJ. Optimizing bone metabolism in osteoporosis: Insight into the pharmacologic profile of strontium ranelate. Osteoporos Int 2003;14 Suppl 3:S9-12.
16. Norman D, Reis D, Zinman C, Misselevich I, Boss JH. Vascular deprivation-induced necrosis of the femoral head of the rat. An experimental model of avascular osteonecrosis in the skeletally immature individual or Legg-Perthes disease. Int J Exp Pathol 1998;79:173-81.
17. The classic. The first stages of coxa plana by Henning Waldenström 1938. Clin Orthop Relat Res 1984;191:4-7.
18. Meek MF, Van Der Werff JF, Nicolai JP, Gramsbergen A. Biodegradable p(DLLA-epsilon-CL) nerve guides versus autologous nerve grafts: Electromyographic and video analysis. Muscle Nerve 2001;24:753-9.
19. Koob TJ, Pringle D, Gedbaw E, Meredith J, Berrios R, Kim HK. Biomechanical properties of bone and cartilage in growing femoral head following ischemic osteonecrosis. J Orthop Res 2007;25:750-7.
20. Miao D, Scutt A. Histochemical localization of alkaline phosphatase activity in decalcified bone and cartilage. J Histochem Cytochem 2002;50:333-40.
21. Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, *et al.* Standardized nomenclature, symbols, and units for bone histomorphometry: A 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 2013;28:2-17.
22. Hamdy NA. Strontium ranelate improves bone microarchitecture in osteoporosis. Rheumatology (Oxford) 2009;48 Suppl 4:iv9-13.
23. Kim HK, Aruwajoye O, Stetler J, Stall A. Effects of non-weight-bearing on the immature femoral head following ischemic osteonecrosis: An experimental investigation in immature pigs. J Bone Joint Surg Am 2012;94:2228-37.
24. Kim HK, Aruwajoye O, Du J, Kamiya N. Local administration of bone morphogenetic protein-2 and bisphosphonate during non-weight-bearing treatment of ischemic osteonecrosis of the femoral head: An experimental investigation in immature pigs. J Bone Joint Surg Am 2014;96:1515-24.
25. Vandermeer JS, Kamiya N, Aya-ay J, Garces A, Browne R, Kim HK. Local administration of ibandronate and bone morphogenetic protein-2 after ischemic osteonecrosis of the immature femoral head: A combined therapy that stimulates bone formation and decreases femoral head deformity. J Bone Joint Surg Am 2011;93:905-13.
26. Fan L, Zhang C, Yu Z, Shi Z, Dang X, Wang K. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and osteogenesis in rabbit femoral head osteonecrosis. Bone 2015;81:544-53.
27. Chen YP, Chen WC, Wang KC, Chen CH. Effectiveness of synovial fluid mesenchymal stem cells embedded in alginate beads for treatment of steroid-induced avascular necrosis of the femoral head. J Orthop Sci 2014;19:657-66.
28. Abrahamsen B, Grove EL, Vestergaard P. Nationwide registry-based analysis of cardiovascular risk factors and adverse outcomes in patients treated with strontium ranelate. Osteoporos Int 2014;25:757-62.
29. Nardone V, Zonefrati R, Mavilia C, Romagnoli C, Ciuffi S, Fabbri S, *et al. In vitro* effects of strontium on proliferation and osteoinduction of human preadipocytes. Stem Cells Int 2015;2015:871863.