Int. J. Oral Maxillofac. Surg. 2011; 40: 413–418
doi:10.1016/j.ijom.2010.10.015, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Research Paper
Osteobiology

Effect of icariin on bone formation during distraction osteogenesis in the rabbit mandible

H. Wei[1], L. Zili[1], C. Yuanlu[2], Y. Biao[1], L. Cheng[1], W. Xiaoxia[1], L. Yang[1], W. Xing[1]

[1]Center of Orthognathic Surgery, Peking University School of Stomatology, Beijing, People's Republic of China; [2]Key Laboratory of Pharmacology of Traditional Chinese Medical Formulae, Ministry of Education (Tianjin University of Traditional Chinese Medicine), Tianjin, People's Republic of China

H. Wei, L. Zili, C. Yuanlu, Y. Biao, L. Cheng, W. Xiaoxia, L. Yang, W. Xing: Effect of icariin on bone formation during distraction osteogenesis in the rabbit mandible. Int. J. Oral Maxillofac. Surg. 2011; 40: 413–418.

Abstract. The aim of this study was to evaluate the effect of icariin on bone formation during mandibular distraction. 40 Rabbits were randomly divided into experimental and control groups. Mandibular distraction was performed 5 days after unilateral mandibular osteotomy using a custom-made external distractor at a rate of 0.5 mm/12 h for 10 days. From the first day of distraction, icariin (2.5 mg/kg·day) was orally administered to the experimental group and placebo to the controls. 10 Rabbits were killed at the end of weeks 2 and 4 of the consolidation phase. The distracted hemimandible was harvested and newly formed bone was evaluated by soft radiography, histology and bone histomorphometry. Regenerated bone was evaluated for bone mineral density by dual-energy X-ray absorptiometry. The experimental group had fewer radiolucent areas on soft radiography. Bone mineral density of regenerated bone was higher in the experimental than in the control group at 2 and 4 weeks. At 4 weeks, the experimental group had greater volumes of new bone, higher trabecular number, and less trabecular separation than the controls. Oral administration of icariin could promote bone formation during mandibular distraction osteogenesis and might be a promising method for shortening the course of distraction osteogenesis.

Keywords: icariin; distraction osteogenesis; bone mineral density; bone histomorphometry.

Accepted for publication 14 October 2010
Available online 16 November 2010

Distraction osteogenesis is a common surgical technique that can successfully restore bone defects or lengthen short or hypoplasia bone without the need for bone grafts. Since its first application in the field of oral and maxillofacial surgery by Mccarthy in 1992[11], the technique has been used for a variety of craniofacial deformities, including micrognathia, temporomandibular joint ankylosis, obstructive sleep apnea syndrome and bone defects after tumorectomy[8,19,26]. The technique is effective in generating new bone by gradually applying tensile strain to an osteotomy site using a distractor. The distraction gap fills with immature bone that differentiates into normal bone after a consolidation phase[7,14]. Distraction osteogenesis has many advantages over traditional orthopedic techniques, including not requiring bone grafting and simultaneous expansion of the surrounding soft tissue[28].

Despite the advantages and the general clinical acceptance of distraction osteogenesis, a long consolidation period is required for complete ossification in the distraction gap. Patients are at risk of complications during this time, such as pain, refracture, infection or nonunion[12],

0901-5027/040413 + 06 $36.00/0

and suffer a greater psychological and economical burden. Many studies have focused on the promotion of new bone formation to shorten the course of distraction osteogenesis. Physical means have included pulsed electromagnetic fields, low-intensity ultrasound and electrical stimulation[9,18]. Interventional methods, such as transplantation of osteoblast-like cells or bone marrow to the distraction site, injection of growth factors or platelet rich plasma and gene therapy have also been applied to accelerate the maturation of the regenerated bone[17,22]. Some noninterventional methods, such as administration of calcitonin, alendronate and zoledronic acid have also shown promising results[15,20].

Epimedium brevicornum maxim (*Yin Yang Huo*, horny goat weed) has been used as a medicinal herb to treat fractures, bone and joint diseases, and gonad dysfunction in traditional Chinese medicine for thousands of years[29]. Icariin ($C_{33}H_{40}O_{15}$, molecular weight: 676.67), is a major and indicative ingredient isolated from *Epimedium brevicornum maxim* that has a wide range of pharmacological and biological effects[27]. Its structure shown in Fig. 1. Icariin-containing drugs exert beneficial effects in the treatment of osteoporosis and prevention of osteonecrosis, through stimulation of bone formation and inhibition of bone resorption[29,30]. Application of a single component of icariin to a rat hypoandrogenism model showed that icariin might promote bone formation and reduce bone resorption. Icariin has therapeutic potential in the management of hypoandrogenism[31].The application of icariin during distraction osteogenesis to promote new bone formation has yet not been reported.

In this study, the authors hypothesize that icariin might be a suitable candidate for a non-interventional method to promote bone formation during distraction osteogenesis. The aim of this study is to determine the effect of icariin on bone formation during mandibular distraction by soft radiography, histology, bone histomorphometry and dual-energy X-ray absorptiometry (DEXA).

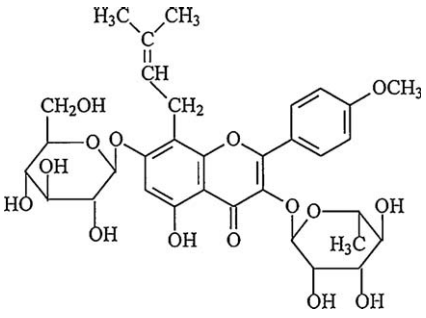

Fig. 1. Structure of icariin.

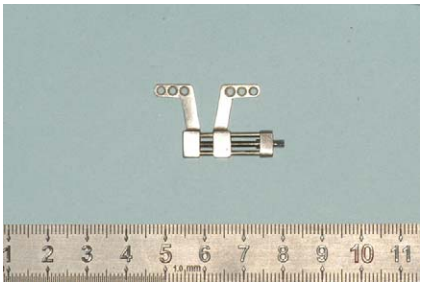

Fig. 2. Custom-made titanium distractor.

Materials and methods

40 Male Japanese white rabbits, 4–5 months old and weighing 3.0–3.5 kg at the beginning of the experiment, were purchased from the Peking University Department of Laboratory Animal Science (Beijing, China). The housing, care, and experimental protocols were approved by the University's Laboratory Animal Care and Use Committee. The titanium distractors used in this experiment were custom-made and could be fixed to the lateral side of the mandible with three self-tapping titanium screws on each side (Fig. 2). Icariin of 98% purity was commercially purchased from the Tcm Institute of Chinese Materia Medica, NanJing, China.

Surgical procedures

Identical surgical techniques, performed by the same surgeon, were used on every animal. Surgery was performed under general anesthesia with an intravenous injection of 30 mg/kg pentobarbital sodium (Sigma, USA) and subcutaneous administration of 2% lidocaine at the surgical site. The submandibular and cheek hairs were shaved. A submandibular curve incision was made in each rabbit along the inferior border of the right mandible and the periosteal flap was raised from the inferior surface of the mandible body. Unilateral osteotomy was performed with a water-cooled fissure burr just between the first premolar and the mental foramen. The osteotomy was completed using a small osteotome. Custom-made external titanium distractors were applied and secured to the mandible with self-tapping screws. The distractor was activated during the operation to confirm the successful osteotomy, then rotated back to the original position. Subcutaneous tissues and skin were closed in layers (Fig. 3).

The animals were housed in separate cages in a recognized animal holding facility on a 12-h light/dark schedule and were fed *ad libitum*. 40,000 IU/kg penicillin and 80,000 IU/kg streptomycin were administered intramuscularly daily for 5 days. The animals' body weights were monitored every week postoperatively to adjust the administration of icariin.

Mandibular distraction and icariin administration

All rabbits were randomly divided into two groups (experimental and control), with 20 rabbits in each. Following a 5-day latency

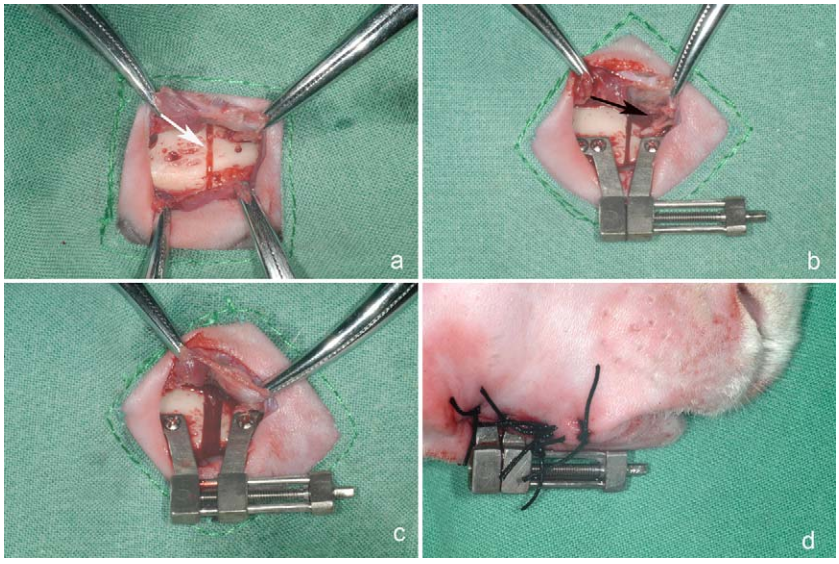

Fig. 3. The surgical procedure: (a) osteotomy line (white arrow); (b) distractor fixed to the mandible, inferior alveolar neurovascular bundle (black arrow); (c) activation of the distractor to prove the completion of the osteotomy; (d) position of the distractor after surgery.

period, the distraction was performed at a rate of 0.5 mm twice daily for 10 days, to a total length of 10 mm. From the first day of the distraction, icariin (2.5 mg/kg·day) was orally administered to the experimental group and a placebo to the control group until the animals were killed. At the end of the second and the fourth week of the consolidation phase, 10 animals from each group were killed with an intravenous overdose of 100 mg/kg pentobarbital. The distracted hemimandible was harvested and the surrounding soft tissue was excised.

The distances achieved were measured using a sliding caliper (mean of middle, superior and inferior boundaries). The newly formed bone in the distracted gap was evaluated by soft radiography, histology, bone histomorphometry and DEXA.

Radiographic evaluation and DEXA

The mandibles were cut into left and right halves. Lateral soft X-ray (REGIUS PureView System, Konica Minolta, Japan) radiographs were taken. The bone mineral density (BMD) of the newly formed bone was determined using DEXA (Lunar Prodigy, General Electric, USA). The distracted mandible was scanned and analyzed using the special animal program provided by the manufacturer. All experimental data were sampled three times.

Histology and histomorphometric analysis

The distracted bones and the adjacent original bone were cut out en bloc and split longitudinally along the median axial plane. The specimens were fixed in phosphate-buffered 10% formal saline for 24 h, decalcified with 14% EDTA for 5–6 weeks, then dehydrated in an ethanol series and embedded in paraffin. Sections of 5 μm were cut, one section was selected per 50 μm, five selected sections of each specimen were included and stained with hematoxylin–eosin. Digital images of histological sections were captured using a light microscope (OP750, OLYMPUS, Japan) and histomorphometric measurements were made using a computerized image analysis system (Leica QWin Plus, Leica, Germany) for blind quantification of new bone formation in the distracted gap. The nomenclature and calculations were in accordance with the American Society of Bone and Mineral Research Histomorphometry Nomenclature Committee[16].

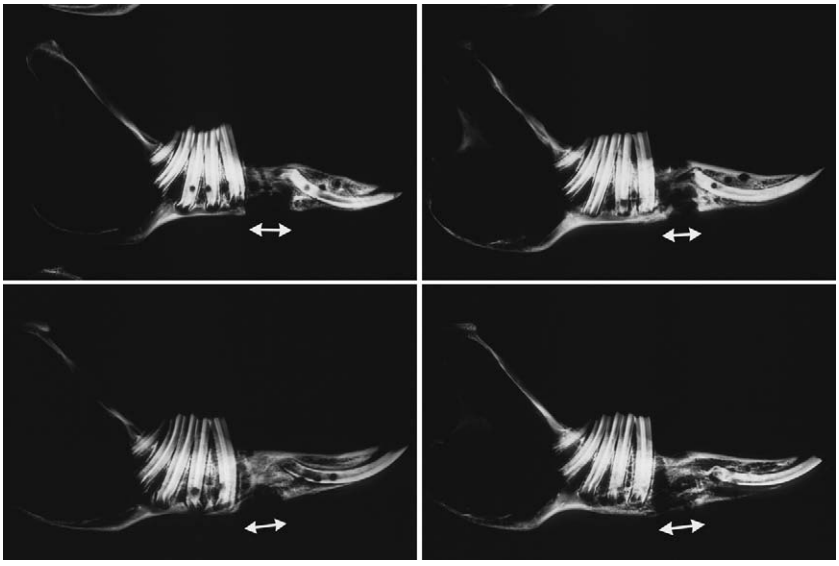

Fig. 4. Representative soft X-ray radiographs from each group. The experimental group had fewer radiolucent areas. (Above) 2 weeks of consolidation; (below) 4 weeks of consolidation; (left) experimental group; (right) control group. White arrow shows the distraction gap.

Statistical analysis

Mean values and standard errors of the mean are presented. A one-way analysis of variance (ANOVA) was used to calculate differences of the distracted length and an unpaired t test (two tailed) was used to compare differences in BMD and histomorphometric parameters of each group. All statistical analyses were carried out using SPSS software 11.0 (SPSS, Munich, Germany). The level of statistical significance was set at $P < 0.05$.

Results

Clinical evaluation

Both the surgical procedure and the distraction period were well-tolerated by all rabbits. The lengthening of the mandible caused malocclusion and overgrowth of upper and lower incisors. In order not to affect mastication and protect the gingiva, the incisors were shortened by regular grinding. Gross postdistraction specimens clearly demonstrated evidence of callus formation at the site of distraction in both groups. The average distraction length for all rabbits was 9.66 ± 0.30 mm.There were no statistical differences in the mean distraction length between the experimental and the control groups (ANOVA, $F = 0.890$; df = 39; $P > 0.05$).

Examination of soft X-ray radiographs

Representative soft X-ray radiographs of each group are shown in Fig. 4. At 2 weeks, the soft X-ray radiographs of both groups showed radiolucent and slightly radio-opaque areas in the distracted gaps. The radiodensity in the experimental group appeared to be higher than in the control group, although it was not significant. At 4 weeks, radio-opacity was observed in most of the distracted gaps in both groups and was more pronounced in the experimental group than that in the control group (Fig. 4).

Bone densitometry

The BMD of the regenerated bone is shown in Fig. 5. Analysis of the bone in the distraction gap revealed that BMD in the experimental group was higher than in the control group, at 2 weeks (0.237 ± 0.040 g/cm^2 vs. 0.190 ± 0.057 g/cm^2, t test, $t = 2.130$; df = 18; $P < 0.05$) and at 4 weeks (0.295 ± 0.045 g/cm^2 vs. 0.245 ± 0.058 g/cm^2, t test, $t = 2.185$; df = 18; $P < 0.05$). With the administration of icariin, the BMD of regenerated bone in the distraction gap significantly increased, by 25% and 20% after 2 and 4 weeks of consolidation, respectively.

Histology and histomorphometric analysis

After 2 weeks of consolidation, sparse trabeculae surrounded by proliferating osteoblasts arranged along the direction of distraction could be seen in the distracted gaps of both groups. Osteoid could be observed on the surface of newly formed trabecular bone. Bone fusion could clearly be seen between the regenerated bone and

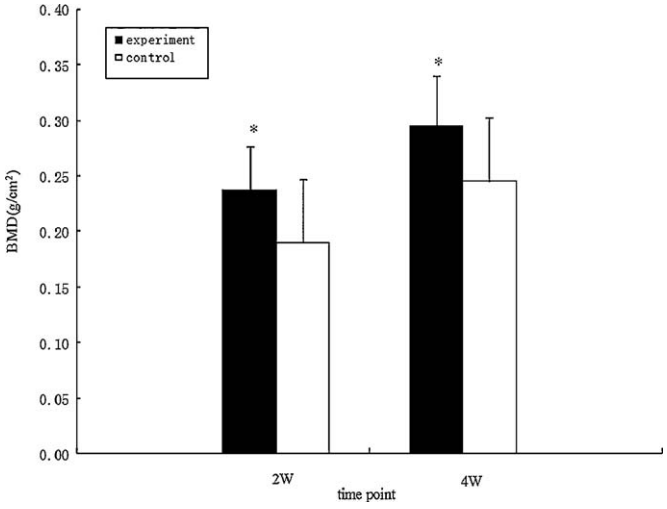

Fig. 5. BMD measured by DEXA. *$P < 0.05$ for the experimental group vs. the control group. BMD, bone mineral density; 2W, 2 weeks; 4W, 4 weeks of consolidation.

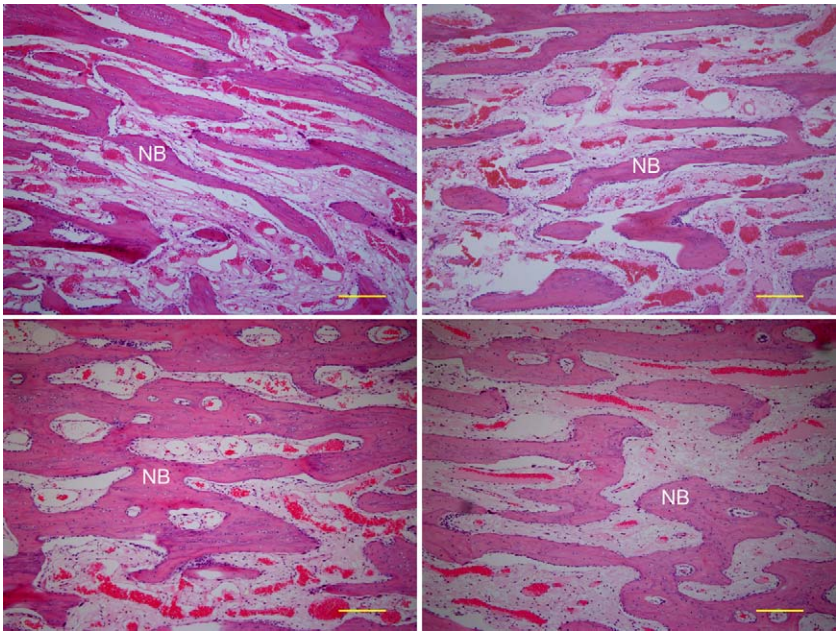

Fig. 6. Histological presentation of regenerated bone from each group. (Above) 2 weeks of consolidation; (below) 4 weeks of consolidation; (left) experimental group; (right) control group. NB, newly formed trabecular bone. Hematoxylin–eosin ×100, bar equals 200 μm. All figures represent similar zones (zone of sclerosis) and depths of the regenerate.

the adjacent original bone. At the end of 4 weeks of consolidation, the trabecular zone increased and became thicker, and the boundary between the regenerate bone and the adjacent original bone and the adjacent original bone became unclear. Newly formed trabeculae, aligned parallel to the axis of lengthening, became joined by bridges (Fig. 6). More cartilaginous islands could be observed in the control group than in the experimental group at both time points.

At 4 weeks, histomorphometric analysis revealed that the new bone volume (37.47 ± 9.03 vs. 25.96 ± 5.89, t test, $t = 3.337$; df = 18; $P < 0.05$) and trabecular number (9.78 ± 3.88 vs. 6.77 ± 2.19, t test, $t = 2.137$; df = 18; $P < 0.05$) in the experimental group were significantly increased over the control group ($P < 0.05$). Trabecular separation of the experimental group was significantly decreased relative to the control group (74.80 ± 33.53 μm vs. 119.09 ± 34.98 μm, t test, $t = -2.891$; df = 18; $P < 0.05$). Statistically significant differences were not detected in trabecular thickness between the two groups (t test, $t = 0.441$; df = 18; $P > 0.05$). At 2 weeks, all the parameters mentioned above showed no significant difference between the experimental group and the control group ($P > 0.05$) (Table 1).

Discussion

Over the past 20 years, animal models of mandibular distraction including rat, rabbit, minipig, dog and monkey have been created and used in different studies. The rabbit model of mandible distraction is well-established and extensively used because of its simple surgery protocols, low-cost, moderate jaw size and occurrence patterns and because its peak bone mass is similar to that of human bone[4]. The protocols for operative technique, waiting period, and rate and speed of lengthening used in this study were based on the literature[4,28]. The results demonstrate that the protocol is reliable and well-tolerated. The animals were killed at the end of 2 or 4 weeks of the consolidation phase because previous studies found that the major significant differences in histology and BMD of the regenerated bone promoted by facilitation methods occurred during the first 2–4 weeks[1,10].

DEXA is extensively used for the clinical evaluation of BMD, and has been used to analyze the BMD of regenerated bone in many distraction studies. In the current

Table 1. Results of histomorphometric analysis.

Parameters	New bone volume (%)	Trabecular thickness (μm)	Trabecular number (#/mm)	Trabecular separation (μm)
2W				
Experimental	26.35 ± 9.98	38.03 ± 10.92	6.99 ± 2.23	119.28 ± 52.39
Control	21.78 ± 7.49	35.68 ± 12.79	6.37 ± 2.13	139.50 ± 56.81
4W				
Experimental	37.47 ± 9.03*	43.41 ± 18.17	9.78 ± 3.88*	74.80 ± 33.53*
Control	25.96 ± 5.89	40.46 ± 11.01	6.77 ± 2.19	119.09 ± 34.98

* $P < 0.05$.

study, DEXA analyses showed that administration of icariin significantly increased the BMD of the regenerated bone at the end of 2 and 4 weeks of consolidation. Histomorphometric analysis afforded some understanding of the dynamics of bone deposition, bone resorption and bone structure. The four parameters used in this study, new bone volume, trabecular number, trabecular thickness and trabecular separation, are reflections of bone structure[24]. By the end of 4 weeks of consolidation, new bone volume and trabecular number increased, and trabecular separation decreased in the experimental group compared with the control, suggesting icariin improved the structure of the regenerated bone. By the end of 2 weeks of consolidation, the histomorphometric results showed no significant difference between the two groups, indicating that at this consolidation time point, icariin had no effect on structure of the new bone. The result of DEXA showed increased BMD in the experimental group at 2 weeks. The different results from the two different measurements may be caused because DEXA evaluates the BMD by detecting calcium, phosphorus and other mineral content in bone, and does not reflect the morphological features of bone[23]. Icariin might still promote the deposition of calcium, phosphorus and other inorganic salts in bone, resulting in the improvement of bone density. The results of histomorphometric and DEXA analysis suggested that the administration of icariin during mandibular distraction may need to continue through 4 weeks of the consolidation phase to obtain improvements in both BMD and bone structure.

In vitro studies demonstrated that icariin stimulates the proliferation of rat bone marrow stromal cells, enhances the osteogenic differentiation of marrow stromal cells and osteoblasts by promoting alkaline phosphatase activity, osteoalcin secretion and calcium deposition level[3,6]. Icariin can inhibit the growth and differentiation of hemopoietic cells from which osteoclasts are formed[2]. Icariin and its metabolites, such as icartin, suppress the differentiation activity of osteoclasts and thus regulate bone resorption via its effects on osteoprotegerin (OPG) and receptor activator of NF-kB Ligand (RANKL) expression and increase the ratio of OPG/RANKL[5,6]. In in vivo studies, icariin treatment improved the condition of reproductive organs and increased the circulating levels of testosterone, and improved the steady-state serum bone Gla-protein (BGP) in male Sprague–Dawley rats after the reproductive system was damaged, suggesting that icariin has testosterone mimetic properties and might have the ability to promote bone formation[31]. Icariin and its glycosides have structural similarities to estrogen and binding capabilities to estrogen receptors[5,29], which may regulate bone remodeling via estrogen receptor pathway within bone cells[21]. In ovariectomized rats, icariin has a definite anti-osteoporotic effect similar to estrogen. Icariin corrected the decreased serum concentration of calcium, phosphorus and E2. The decrease in BMD was suppressed and the biomechanical strength of bone significantly increased by treatment with icariin[13]. Although the exact role of icariin during mandibular distraction has not been elucidated, the promotional effect of icariin may include the mechanisms mentioned above.

This study has limitations that await further studies. The dose of icariin used in this experiment was based on data from preliminary studies[29,30,31]. Only one dose was applied, and more detailed experiments are needed to identify the optimal dose to accelerate bone formation. Other studies have demonstrated that histomorphometric parameters have relevance to biomechanical properties[25], and icariin can increase BMD and improve the bone structure of formed bone at the end of 4 weeks of consolidation. It might also be able to promote biomechanical properties of the new bone, but this hypothesis requires further study.

In conclusion, the present study demonstrated that icariin promoted new bone formation during mandibular distraction osteogenesis in a rabbit model. Administration of icariin may be a potential clinical candidate for accelerating the consolidation phase and shortening treatment duration.

Funding

This study is financially supported by National Key Project of Scientific and Technical Supporting Program of China (No. 2007BAI18B04).

Competing interests

None declared.

Ethical approval

Approval for the study was provided by the Institutional Review Board (IRB) of Peking University Health science Center (No. LAB2008-011).

References

1. Aronson J, Shen XC, Gao GG, Miller F, Quattlebaum T, Skinner RA, Badger TM, Lumpkin Jr CK. Sustained proliferation accompanies distraction osteogenesis in the rat. J Orthop Res 1997: **15**: 563–569.
2. Chen KM, Ge BF, Liu XY, Ma PH, Lu MB, Bai MH, Wang Y. Icariin inhibits the osteoclast formation induced by RANKL and macrophage-colony stimulating factor in mouse bone marrow culture. Pharmazie 2007: **62**: 388–391.
3. Chen KM, Ge BF, Ma HP, Liu XY, Bai MH, Wang Y. Icariin, a flavonoid from the herb Epimedium enhances the osteogenic differentiation of rat primary bone marrow stromal cells. Pharmazie 2005: **60**: 939–942.
4. Djasim UM, Wolvius EB, van Neck JW, Weinans H, van der Wal KG. Recommendations for optimal distraction protocols for various animal models on the basis of a systematic review of the literature. Int J Oral Maxillofac Surg 2007: **36**: 877–883.
5. Haiyong Y, Yijia L. Estrogenic effects of two derivatives of icariin on human breast cancer MCF-7 cells. Phytomedicine 2005: **12**: 735–741.
6. Huang J, Yuan L, Wang X, Zhang TL, Wang K. Icariin and its glycosides enhance osteoblastic, but suppress osteoclastic, differentiation and activity in vitro. Life Sci 2007: **81**: 832–840.
7. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 1989: 249–281.
8. Kashiwa K, Kobayashi S, Kimura H, Honda T, Nohara T, Fujiwara H, Hosoya Y, Arai Y. Reconstruction of a severe maxillofacial deformity after tumorectomy and irradiation using distraction osteogenesis and LeFort I osteotomy before vascularized bone graft. J Craniofac Surg 2007: **18**: 1133–1137.
9. Kesemenli CC, Subasi M, Kaya H, Sert C, Buyukbayram H, Arslan H, Necmioglu S. The effects of electromagnetic field on distraction osteogenesis. Yonsei Med J 2003: **44**: 385–391.
10. Li G, Simpson AH, Kenwright J, Triffitt JT. Assessment of cell proliferation in regenerating bone during distraction osteogenesis at different distraction rates. J Orthop Res 1997: **15**: 765–772.
11. McCarthy JG, Schreiber J, Karp N, Thorne CH, Grayson BH. Lengthening the human mandible by gradual distraction. Plast Reconstr Surg 1992: **89**: 1–8.
12. Mofid MM, Manson PN, Robertson BC, Tufaro AP, Elias JJ, Vander Kolk CA. Craniofacial distraction osteogenesis: a review of 3278 cases. Plast Reconstr Surg 2001: **108**: 1103–1114 [discussion 15–17].
13. Nian H, Ma MH, Nian SS, Xu LL. Antiosteoporotic activity of icariin in

ovariectomized rats. Phytomedicine 2009: **16**: 320–326.
14. Pampu AA, Dolanmaz D, Tuz HH, Avunduk MC, Kisnisci RS. Histomorphometric evaluation of the effects of zoledronic acid on mandibular distraction osteogenesis in rabbits. J Oral Maxillofac Surg 2008: **66**: 905–910.
15. Pampu AA, Dolanmaz D, Tuz HH, Karabacakoglu A. Experimental evaluation of the effects of zoledronic acid on regenerate bone formation and osteoporosis in mandibular distraction osteogenesis. J Oral Maxillofac Surg 2006: **64**: 1232–1236.
16. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 1987: **2**: 595–610.
17. Qi M, Hu J, Zou S, Zhou H, Han L. Mandibular distraction osteogenesis enhanced by bone marrow mesenchymal stem cells in rats. J Craniomaxillofac Surg 2006: **34**: 283–289.
18. Sakurakichi K, Tsuchiya H, Uehara K, Yamashiro T, Tomita K, Azuma Y. Effects of timing of low-intensity pulsed ultrasound on distraction osteogenesis. J Orthop Res 2004: **22**: 395–403.
19. Schwartz HC, Relle RJ. Distraction osteogenesis for temporomandibular joint reconstruction. J Oral Maxillofac Surg 2008: **66**: 718–723.
20. Sen C, Gunes T, Erdem M, Koseoglu RD, Filiz NO. Effects of calcitonin and alendronate on distraction osteogenesis. Int Orthop 2006: **30**: 272–277.
21. Setchell KD, Lydeking-Olsen E. Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. Am J Clin Nutr 2003: **78**: 593S–609S.
22. Shao Z, Liu B, Peng Q, Liu W, Liu Y, Liu R, Xu Y, Liu L. Transplantation of osteoblast-like cells to the distracted callus in the rabbit mandible. Plast Reconstr Surg 2007: **119**: 500–507.
23. Srivastava M, Deal C. Osteoporosis in elderly: prevention and treatment. Clin Geriatr Med 2002: **18**: 529–555.
24. Takahashi M, Yukata K, Matsui Y, Abbaspour A, Takata S, Yasui N. Bisphosphonate modulates morphological and mechanical properties in distraction osteogenesis through inhibition of bone resorption. Bone 2006: **39**: 573–581.
25. Thomsen JS, Ebbesen EN, Mosekilde L. Predicting human vertebral bone strength by vertebral static histomorphometry. Bone 2002: **30**: 502–508.
26. Wang X, Wang XX, Liang C, Yi B, Lin Y, Li ZL. Distraction osteogenesis in correction of micrognathia accompanying obstructive sleep apnea syndrome. Plast Reconstr Surg 2003: **112**: 1549–1557.
27. Wu H, Lien EJ, Lien LL. Chemical and pharmacological investigations of Epimedium species: a survey. Prog Drug Res 2003: **60**: 1–57.
28. Yonezawa H, Harada K, Ikebe T, Shinohara M, Enomoto S. Effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on bone consolidation on distraction osteogenesis: a preliminary study in rabbit mandibles. J Craniomaxillofac Surg 2006: **34**: 270–276.
29. Zhang G, Qin L, Hung WY, Shi YY, Leung PC, Yeung HY, Leung KS. Flavonoids derived from herbal *Epimedium Brevicornum Maxim* prevent OVX-induced osteoporosis in rats independent of its enhancement in intestinal calcium absorption. Bone 2006: **38**: 818–825.
30. Zhang G, Qin L, Sheng H, Yeung KW, Yeung HY, Cheung WH, Griffith J, Chan CW, Lee KM, Leung KS. Epimedium-derived phytoestrogen exert beneficial effect on preventing steroid-associated osteonecrosis in rabbits with inhibition of both thrombosis and lipid-deposition. Bone 2007: **40**: 685–692.
31. Zhang ZB, Yang QT. The testosterone mimetic properties of icariin. Asian J Androl 2006: **8**: 601–605.

Address:*Wang Xing*
Center of Orthognathic Surgery
Peking University School of Stomatology
Zhongguancun South Road 22
Haidian District
Beijing 100081
People's Republic of China
Tel.: +86 010 6211 6655
fax: +86 010 6211 0880
E-mail: kqwx@vip.sina.com