Original Article

The potential use of phytoestrogen containing the herb, Pueraria mirifica, for bone healing in osteoporotic monkeys

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Abstract

Within the last decade, the positive effects of *Pueraria mirifica* on osteoporosis have been established in rats and monkeys. However, the effect of *P. mirifica* on bone healing has not been elucidated. In this study, post-menopausal osteoporotic monkeys were subjected to an iliac crest biopsy, divided into two groups (5 animals/group), and fed daily with standard monkey diet alone (PMP0 group) or mixed with 1,000 mg/kg body weight of *P. mirifica* powder (PMP1000 group) for 16 months. The progression of the bone healing was continuously assessed by X-ray radiography, and three individuals from each group were selected for a computed tomographic (CT) scan at 0, 8 and 16 months. The individual from each group that showed the greatest progression of bone healing, based upon the CT image, was selected for a second biopsy of the right ilium after 16 months of treatment and histological changes determined. The perimeter and area measured by the X-ray radiograph were significantly decreased earlier in the PMP1000 group compared to the PMP0 group, and the significant differences of the perimeter between the groups were detected in month 3, 6 and 8. The 3D-CT scan showed a progressive bone healing in one PMP1000 individual, while histological examination indicated a lower number of fibrocartilage cells and a higher amount of new bone formation compared to the PMP0 monkey. In conclusion, PMP treatment could accelerate the progression of bone fracture healing in naturally postmenopausal osteoporotic monkeys, in addition to the previously reported amelioration of bone loss.

Keywords: Bone mineral contents, Estrogen deficiency, *Macaca fascicularis*, Osteoporosis, *Pueraria mirifica*, X-ray

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Introduction

Bone fractures are a common orthopedic problem in which the continuity of the respective bone is completely or partially disrupted. Indeed, almost half of all fractures are related to osteoporosis, which is characterized by a low bone mass and deterioration of the bone microarchitecture (Kanis et al., 2013). It has been reported that osteoporotic fractures cause a higher mortality and morbidity than cancers (except lung cancer), particularly hip fractures that cause 10–20% increased mortality in age-matched women (Pisani et al., 2016). Currently, osteoporotic fractures are an increasing public health and socioeconomic problem and cost about $20 billion per year in the United States (Cummings and Melton, 2002) and €30.7 billion in Europe (Johnell and Kanis, 2006). The incidence of osteoporotic fractures is growing significantly according to the global demographic trend. The number of osteoporotic hip fractures reached 1.7 million in 1990 and has been projected to reach 21 million in 2050 (Cummings and Melton, 2002).

It is well known that estrogen deficiency is a major risk factor of osteoporosis (Ettinger, 2003). Thus, estrogen or estrogen-like compounds are widely used for the prevention and therapeutics of osteoporosis in postmenopausal women (Beil et al., 2010). Estrogen deficiency or supplementation also directly affects the bone healing process (Tella and Gallagher, 2014). For example, estrogen deficient mice showed an impaired periosteal callus formation, diminished chondrocytes, less mineralization, and a thinner and more porous bone cortex compared to control mice. However, when they were treated with estrogen, the fracture healing was progressed by increasing the chondrocyte area, stimulating mineralization and giving a thicker cortex (Tella and Gallagher, 2014). This is supported by a study in humans that noted that the lack of 17β-estradiol in menopausal women promoted the development of fat tissue from bone marrow cells rather than the osteogenic lineage (Justesen et al., 2001). Although estrogen seems to be beneficial for the treatment of both bone healing and osteoporosis, it also has adverse side effects, such as the promotion of estrogen-dependent cancers and diseases (Gambacciani et al., 2003).

Pueraria mirifica is a leguminous Thai plant belonging to the Family Leguminoceae. Its tuberous roots contain at least 17 phytoestrogenic substances including genistin, genistein, daidzin, daidzein, puerarin and miroestrol (Malaivijitnond, 2012), and have been used in Thai folklore medicine for the rejuvenating qualities in aged women and men for a century. In the past decade, its anti-osteoporotic effects have also been widely tested (Urasopon et al., 2007; Urasopon et al., 2008; Malaivijitnond, 2012; Tiyasatkulkovit et al., 2012; Tiyasatkulkovit et al., 2014; Kittivanichkul et al., 2016[a]; Suthon et al., 2016[a]). *P. mirifica* and its phytoestrogen contents stimulated bone formation and suppressed bone resorption both *in vitro* (Tiyasatkulkovit et al., 2012) and *in vivo* (Urasopon et al., 2007; Urasopon et al., 2008; Suthon et al., 2016[a]) in rodent models. Because rats are lacking in the Haversian system in the cortical bone, the anti-osteoporotic effects of *P. mirifica* were tested further in non-human primate models. Recently, the therapeutic effects of *P. mirifica* have been confirmed in macaques (Kittivanichkul et al., 2016[a]), where *P. mirifica* could ameliorate the loss of cortical bone mass in naturally postmenopausal cynomolgus monkeys, especially at the diaphysis site. Apart from the effect on bone mass, P. mirifica also improved the bone structure by decreasing the endosteal circumferences and increasing the cortical thickness at the diaphysis site of both the radius and tibia.

With respect to the positive effect of *P. mirifica* on osteoporosis in rodents and in non-human primates, its efficacy on bone fracture healing has been raised. Although the Food and Drug Administration (USFDA) has approved many anti-osteoporotic drugs for osteoporotic patients, they have undesirable side effects on bone healing. For example, alendronate, an anti-bone resorptive agent that has been widely used for osteoporosis since it can increase bone density, has been reported to have a negative impact on bone healing (Kates and Ackert-Bicknell, 2016). Moreover, in osteoporotic or osteopenia patients who had spontaneous nonspinal fractures, alendronate therapy resulted in the delay or prevention of bone fracture healing due to the severe suppression of bone turnover (Odvina et al., 2005). Although osteoporosis can be induced in many animal species, spontaneous fractures with no adequate trauma can only be seen in non-human primates (Egermann et al., 2005). Besides, osteoporosis naturally and consequently occurs only in macaques and humans (Egermann et al., 2005). Thus, this study aimed to test whether *P. mirifica*, which had positive effects on osteoporosis, could accelerate the bone fracture healing process in osteoporotic monkeys.

Materials and Methods

Animals: In an attempt to mimic the bone fracture healing process in osteoporotic animals, naturally postmenopausal monkeys that showed bone loss were selected for this study. Ten female cynomolgus monkeys (*Macaca fascicularis*) which were (i) more than 20 years old, (ii) amenorrhea for at least 1 year and (iii) had total bone mineral content (BMCs) at the radius and tibia of between -0.5 to -1 standard deviation (SD) compared to 33 premenopausal monkeys (Fig. 1), were selected for this experiment. The premenopausal monkeys that were selected as a reference of normal BMCs in this study were fully mature females with at least three consecutive regular menstrual cycles. They were housed in a group cage (4–8 animals/cage), which allowed them to move freely, at the Krabok-Koo Wildlife Breeding Center, Chachoengsao Province, Thailand. The post-menopausal monkeys were housed in individual cages at the Primate Research Unit, Chulalongkorn University, Thailand. The monkeys were exposed to a 12:12 h light: dark cycle at ambient temperature. Monkeys were fed daily with monkey chow (Perfect Companion Group. Co., Ltd. Samut Pakran, Thailand) in the morning (09:00–10:00 am) and fresh fruit supplemented in the afternoon (01:00–02:00 pm) and water *ad libitum*. All animal procedures were approved by Faculty of Science, Chulalongkorn University Animal Care and Use Committee (CU-ACUC: Protocol Review no. 1123015, August 8, 2011).

Animal health for each animal was checked daily by veterinary staff and animal caretakers.

Plant materials: The dried *Pueraria mirifica* Airy Shaw & Suvatabandhu powder (PMP) (lot no. 141023) was kindly provided by Dr. I. Sandford Schwartz (Smith Naturals Co., Ltd., Bangkok, Thailand). The tuberous roots of *P. mirifica* were collected from Chiang Mai, northern Thailand and a voucher specimen has been kept at the Professor Kasin Suvatabhandhu Herbarium Thailand (voucher specimen number BCU011045), Department of Botany, Faculty of Science, Chulalongkorn University, Thailand. The materials were examined for at least two species-specific phytoestrogens; puerarin (a species-specific phytoestrogen in the *Pueraria* plant) and miroestrol (a species-specific phytoestrogen in *P. mirifica* which exhibits the highest estrogenic activity in the plant), before being accepted into the manufacture process. Quality control of the manufactured PMP was done by the high-performance liquid chromatography (Bio-Botanica Inc., Hauppauge, NY, USA). After receiving the PMP, the phytoestrogen contents in the PMP were confirmed again by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as reported previously (Kittivanichkul et al., 2016[a]). The puerarin and miroestrol contents were 18.6 mg/100 g PMP (lot no. 141023) and 233 µg/100 g PMP (lot no. 141023), respectively.

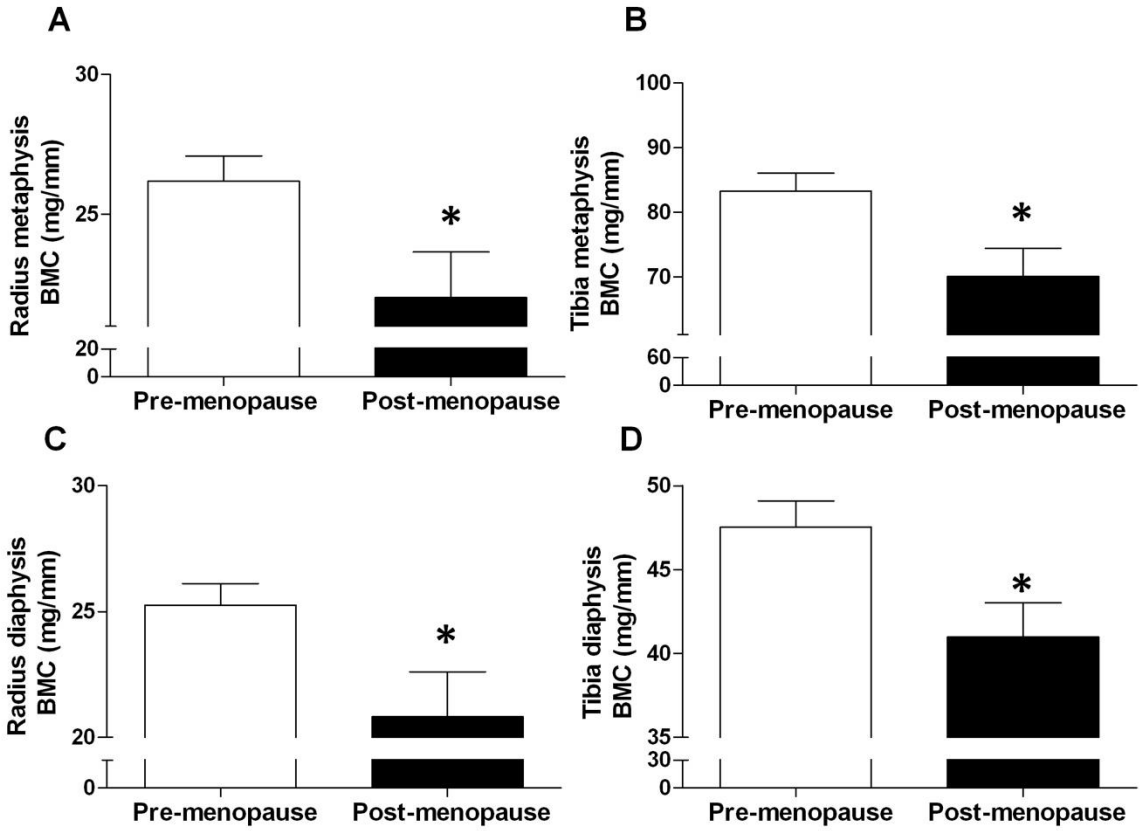

Figure 1 Bone mineral content (BMC) of the (A, C) radius and (B, D) tibia at the (A, B) metaphysis site and (C, D) diaphysis site of pre-menopausal monkeys (n= 33) and post-menopausal monkeys (n = 10) that were selected for the bone healing study. Data are shown as the mean ± S.E.M., and * represents $p< 0.05$ compared with the pre-menopausal monkeys.

Experimental design: Prior to the experiment, all 10 postmenopausal monkeys were subject to an iliac crest biopsy. Two bone fracture defects were created at the right ilium wing (Fig. 2). Three days after the surgical procedure, the monkeys were randomly divided into two groups (n = 5 per group) and fed with standard monkey diet either alone (PMP0) or mixed with PMP at a dose of 1,000 mg/kg body weight (BW)/day (PMP1000) at 08:00–09:00 am for 16 months. The age (29.6 ± 1.8 y and 26.0 ± 2.3 y, respectively) and menopause period (5.8 ± 1.4 y and 7.8 ± 1.2 y, respectively) were not significantly different between the PMP0 and PMP1000 groups. The dose of PMP used in this study was based on its efficacy in retaining cortical bone loss in naturally postmenopausal cynomolgus monkeys reported previously (Kittivanichkul et al., 2016[a]). The dose of PMP treatment was adjusted with the monkey's BW every two months.

Progression of bone healing was accessed by radiography (X-ray) and the determination was performed immediately after the biopsy procedure (month 0) and then at 1, 2, 3, 4, 6, 8, 12 and 16 months afterwards. Three individuals from each PMP0 and PMP1000 group were randomly selected for computed tomography (3D-CT) scans at month 0, 8 and 16. As the bones in aged postmenopausal monkeys are highly porous and fragile, biopsy is difficult to perform and only an individual from each group that showed the greatest progression of bone healing based on the 3D reconstructed CT images was selected for a second biopsy at the right ilium after 16 months of the PMP0

or PMP1000 treatment and examined for histological changes.

Bone biopsy procedure: The monkeys were subcutaneously premedicated with tramadol (4 mg/kg BW; T.P. Laboratories (1969), Bangkok, Thailand) and cefazolin (25 mg/kg BW; Biolab. Co., Ltd., Samutprakarn, Thailand). After that monkeys were anesthetized by intramuscular injection of tiletamine/zolazepam (3 mg/kg BW; Virbac, Nonthaburi, Thailand) and medetomidine hydrochloride (40 µg/kg BW; Vetcare, Joneboro, AR, USA). During the surgical procedure, 4 mg/kg BW carprofen (Zoetis, Pasippany, New Jersey, USA) was subcutaneously injected as an analgesic. The operation was performed under strict aseptic techniques to avoid any complications. The ilium wing was palpated and the biopsied position was marked. Each bone defect was created by a 1 x 1 cm cut from the lateral ilium wing (Fig. 2). The monkeys were intramuscularly injected with tramadol (3 mg/kg BW) and cefazolin (20 mg/kg BW) immediately after surgery and this continued twice a day for 4 days. The monkey's health was checked twice daily for any sign of sickness or complications.

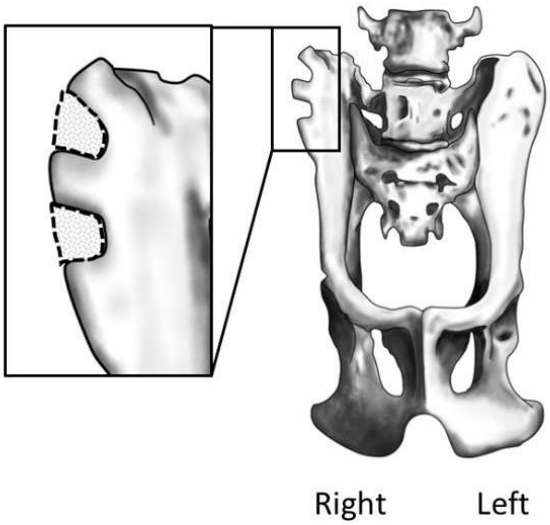

Figure 2 Bone defects were created by a 1 x 1 cm cut from the lateral ilium wing in each monkey. In the magnified insert image, the dashed line and shaded grey area indicate the perimeter and area measurement of the radiography, respectively.

Assessment of bone repair by radiography: Monkeys were anesthetized by intramuscular injection with a mixture of 3 mg/kg BW tiletamine/zolazepam and 40 µg/kg BW medetomidine hydrochloride. They were placed ventrodorsally and the ilium wing was palpated and aligned parallel to the cassette. A 2-cm calibration scale was also placed on the cassette at every exposure. A portable X-ray machine (model PXP-60HF, Poskom, Goyang, Korea) was used with a constant source to image distance of 70 cm while the kilovoltage peak (kVp) and milliampere seconds (mAs) were adjusted to acquire a proper image. After exposure, the X-ray cassette was developed with a digital X-ray reader model FCR Capsula, Fujifilm and the resulting of each bone defect was used to measure the perimeter and area of the damage by a specialist using the Osirix Lite (Version V.7.0.2, Bernex, Switzeland), as a blind assay, and the average value of two bone defects in each monkey was used for further analysis.

Assessment of bone repair by 3D-CT scans: The scan was performed to unveil the anatomical lesion of the ilium at 0, 8 and 16 months after the biopsy using a 64 slice multidetector CT scanner (model Optima CT660, GE, Bangkok, Thailand). All monkeys were immobilized by intramuscular injection of a mixture of 3 mg/kg BW tiletamine/zolazepam and 40 µg/kg BW medetomidine hydrochloride and then were scanned in a supine position on the radiolucent V-pad positioning device. After the pre-scan phase, the region of interest was set to cover the whole area of the pelvic girdle and helical CT images were acquired at 120 kVp, 250 mAs, pitch speed of 0.531 and a slice thickness of 1.25 mm. Subsequently, the CT images were analyzed in the digital imaging and communication in medicine (DICOM) format using the open source workstation viewer (Osirix Lite Version V.7.0.2, Bernex, Switzeland). To measure the bone structure parameters of both two post-operative ilium defects at different time points, the CT images were subjected to 3D multiplanar reconstruction and were revealed under the bone window (WL = 300, WW = 1500). For each ilium defect, the depth was repeatedly measured at the top (0%), center (50%) and lower (100%) position of the lateral height of the defect (see Fig. 4). Moreover, the perimeter and area of each bone defect were also measured.

Bone histology: The second biopsy of the post-treatment right ilium was performed at the end of the treatment period (month 16) as explained above. The bone specimens were trimmed, washed in sterile normal saline solution and fixed in 10% neutral buffer formalin at room temperature. The specimens were decalcified, dehydrated, paraffin embedded, sectioned and stained with hematoxylin and eosin (H&E) by the Vet & Vitro Central Lab, Bangkok, Thailand.

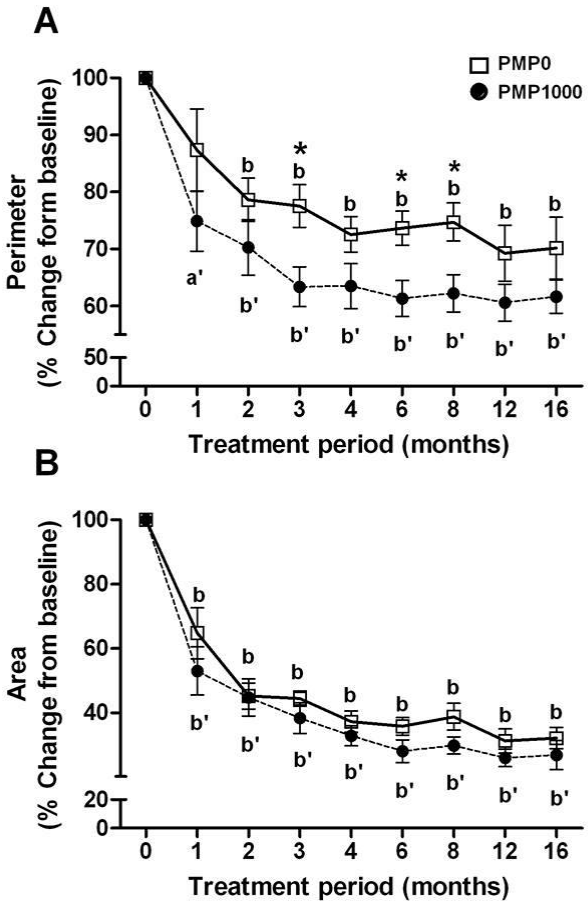

Figure 3 The percent change from baseline of (A) perimeter and (B) area of bone defect, as determined by radiography, during the 16-month experimental period. Open square and closed circle indicate the PMP0 and PMP1000 groups, respectively. Data are shown as the mean ± S.E.M., derived from five monkeys in each group. a, b and a', b' represent $p < 0.05$ and $p < 0.01$ compared between the baseline (month 0) and other time points of the PMP0 and PMP1000 groups, respectively. * represents $p < 0.05$ compared between the PMP0 and PMP1000 groups.

Statistical analysis: The radiography and CT data are presented as the mean ± standard error of the mean (S.E.M). As there was no significant interaction between the time and treatment when tested by two-way repeated measures ANOVA, the effects of time on bone healing in each monkey group, comparing values between month 0 and other time points, were analyzed using a paired t-test. Moreover, the effects of *P. mirifica* treatment compared between the PMP0 and PMP1000 groups at the same time point were analyzed using an unpaired t-test. A level of $p< 0.05$ was accepted as significantly different. Statistical analyses were performed using the IBM SPSS Statistics for windows, version 20.0 software.

Results

Clinical and physical examinations: All monkeys recovered rapidly after surgery and bone biopsy, without any sign of infection or complication. The routine health check by veterinarians found that the monkeys regularly consumed food and drank water. Moreover, the animals remained in good health throughout the 16-month post-biopsy study period.

Decreasing size of the bone defect under PMP treatment: Since the perimeter and area of the bone defects are different when comparing the PMP0 and PMP1000 groups (perimeter = 3.59±0.28 cm for PM0 group and 3.74±0.21 cm for PM1000 group; area = 0.63±0.10 cm^2 for PM0 group and 0.69±0.07 cm^2 for PM1000 group), the values are thus adjusted to percent changes from the baseline (month 0) values. Compared to month 0, the perimeter of the bone defect in the PMP0 group, assessed by X-ray radiography, significantly decreased starting at month 2 of the treatment, while a significant decrease was detected earlier (since month 1) in the PMP1000 group (Fig. 3A). In concordance with the perimeter, the area of the bone defect was significantly decreased starting at month 1 in both groups (Fig. 3B). Comparing the PMP0 and PMP1000 groups, the perimeter of the bone defect of the PMP0 group was significantly higher than the PMP1000 group at month 3, 6 and 8. Although it tended to be higher, no significant differences between the PMP0 and PMP1000 groups were detected in the area throughout the 16-month post-biopsy study period.

The area (Fig. 4A), perimeter (Fig. 4B) and depth (Fig. 4C) of the 3D reconstructed CT images of the bone defect in the PMP0 and PMP1000 groups were not significantly different, considering either the comparison between month 0, 8 and 16 of both monkey groups or between the PMP0 and PMP1000 groups at the same time point. However, the CT image clearly

revealed the progression of bone healing in one monkey of the PMP1000 group where the bone defects were evidently shallower at month 8 and 16 compared to those in the PMP0 group (Fig. 5).

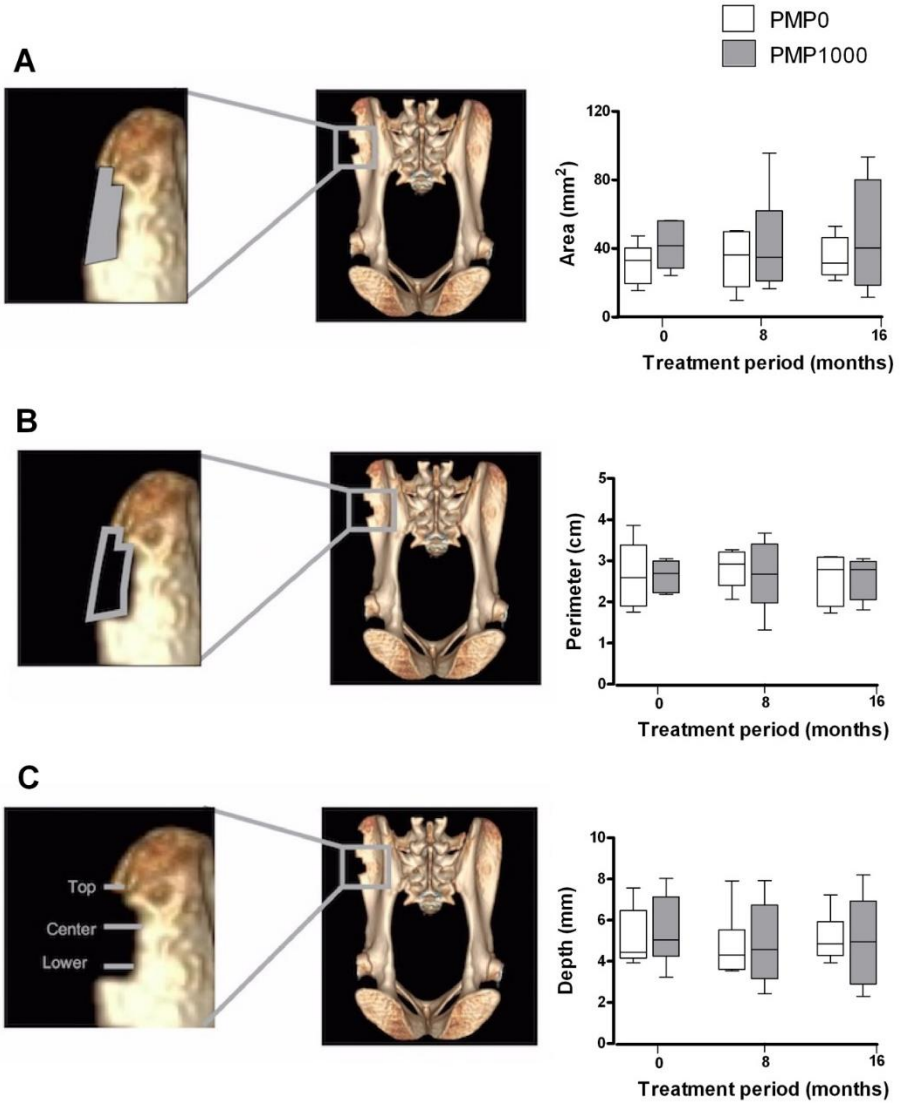

Figure 4 The (A) area, (B) perimeter and (C) depth of the bone defect, as determined by 3D-CT scans at month 0, 8 and 16. Open square at the iliac crest indicates the area of magnification. White and grey boxes in the graphs indicate the PMP0 and PMP1000 groups, respectively. Box plot indicates the median and 25th and 75th percentiles.

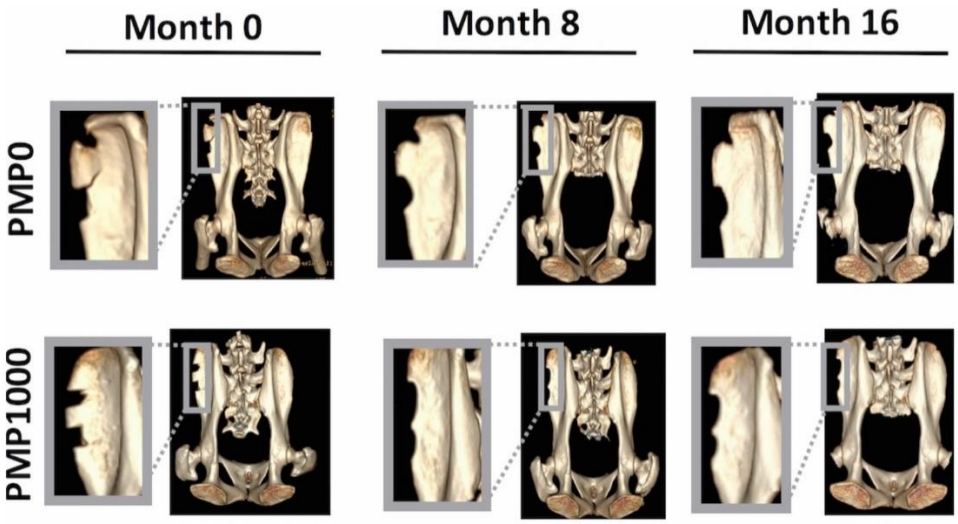

Figure 5 Reconstructed 3D-CT scans at month 0, 8 and 16 of the representative PMP0 (upper panel) and PMP1000 (lower panel) monkeys.

Bone histology: Since the measurements of area, perimeter and depth of bone defect did not indicate the bone healing process, the bone histology was examined. As expected, the PMP0 group showed a thick layer of fibrocartilage cells. In contrast, the PMP1000 group showed a lower number of fibrocartilage cells but a region of new bone formation, as indicated by the presence of chondrocyte cells, was clearly observed (Fig. 6).

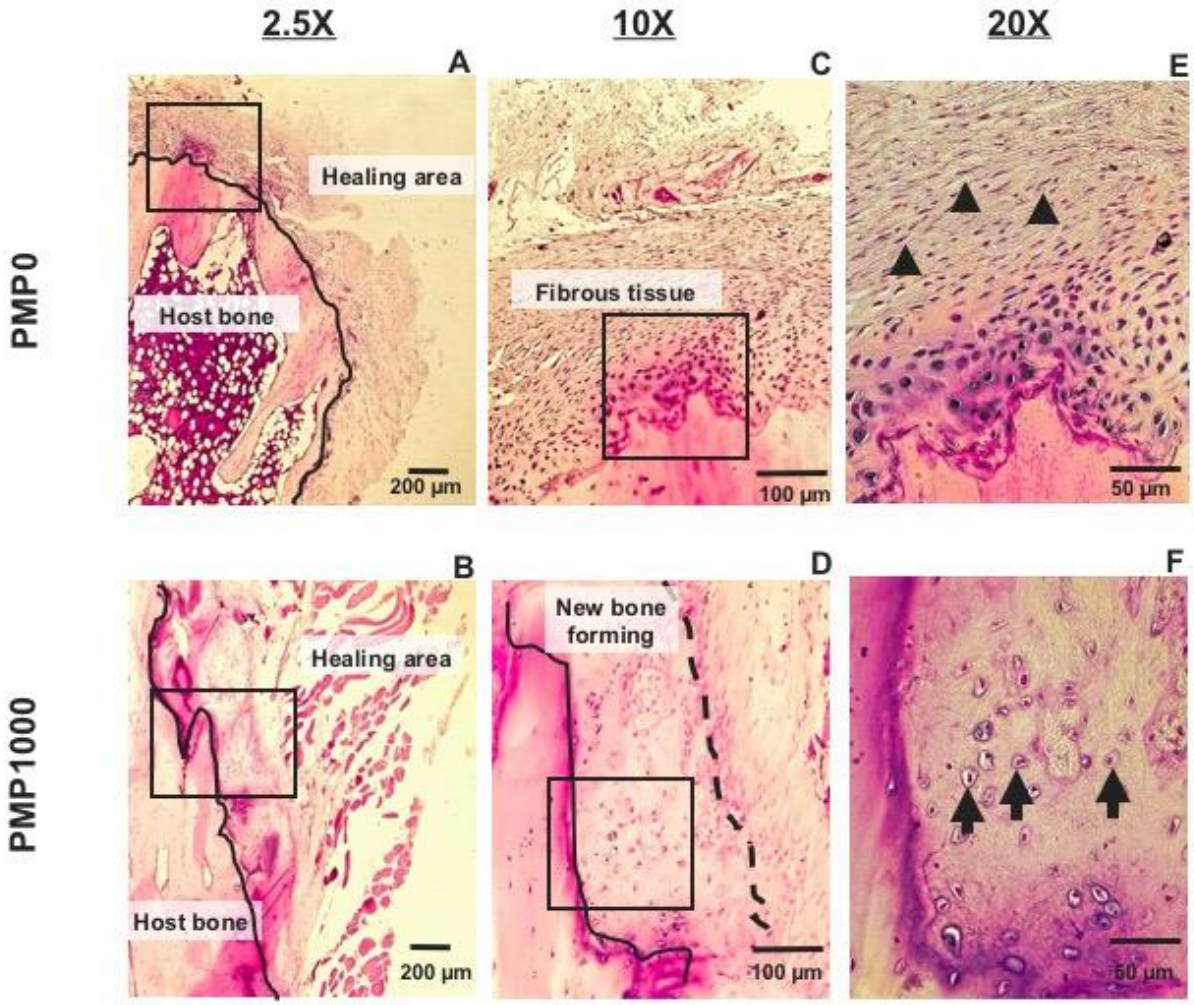

Figure 6 Longitudinal plane histology section (stained with H&E) of the ilium biopsy at month 16 of the (A, C, E) PMP0 and (B, D, F) PMP1000 treated monkeys at (A, B) 25X, (C, D) 100X and (E, F) 200X magnification. In Figs A and B, the host bone area is separated from the healing bone area by the black and thick line and the open squares indicate the area of magnification shown in Figs C and D, accordingly. In Fig C, the thick layer is a fibrous tissue. In Fig D, an area between the thick and dashed lines is a new bone forming area. The open squares in Figs C and D indicate the area of magnification shown in Figs E and F, respectively. The arrow head and arrow in Figs E and F indicate fibrocartilage and chondrocyte cells, respectively. The scale bars are 200 µm, 100 µm and 50 µm for 25X, 100X and 200X magnification.

Discussion

It has been reported that osteoporosis can cause irregular bone healing and affect different stages in the bone healing process depending on the species of animals used. In rats, osteoporosis affects callus formation and mineralization in the early and late stages of bone healing, respectively (Egermann et al., 2005). In osteoporotic sheep, a delay in the tibiae bone healing at callus formation and mineralization is observed as well as altered mechanical properties (Lill et al., 2003). No study of bone fracture healing in osteoporotic monkeys has been conducted yet, this is because it is not easy to recruit naturally osteoporotic monkeys.

The iliac crest was selected for bone biopsy in this study because it was easily accessible and did not require extensive surgery (Malluche et al., 2007). From 99 iliac crest biopsies of osteoporotic patients, complications were observed in only eight (8.1%) cases (Hodgson et al., 1986). Furthermore, the progression of the iliac crest biopsies correlated with the pattern seen for bone repair in the vertebrae, tibiae and femur (Meunier et al., 1973; Dorr et al., 1990; Dorr et al., 1993). Another reason for performing an iliac crest biopsy in this study rather than the usual transiliac crest biopsy was that the transiliac crest biopsy might lead to immobility in the aged osteoporotic monkeys which were used this study. Therefore, the bone defect was created by cutting the lateral site of iliac crest, where the weight bearing or tension is minimal (Malluche et al., 2007).

In this study, PMP at a dose of 1,000 mg/kg BW, which could alleviate cortical bone loss in

naturally post-menopausal cynomolgus monkeys (Kittivanichkul et al., 2016[a]), potentially stimulated the bone healing process as seen in the significant decreases in the perimeter of bone defect in the PMP1000 group compared to the PMP0 group. Our results are in agreement with previous reports on the effect of the phytoestrogens, puerarin, genistein and daidzein, on bone defects in rabbits (Wong and Rabie, 2007; 2009; 2010). These rabbit bone defects were grafted with collagen matrix alone (control) or the same mixed with phytoestrogens. By the early healing stage (day 14) the presence of puerarin, genistein or daidzein in the collagen matrix had significantly increased new bone regeneration by 554%, 520% or 620%, respectively.

Previously, it was reported that genistein and daidzein enhanced osteogenesis while depressing adipogenic differentiation of mesenchymal progenitor cells (Schilling et al., 2014). Likewise, it was shown that genistein and daidzein enhanced the osteogenic activity in mesenchymal and adipocyte stem cells (Strong et al., 2014). Moreover, *in vitro* studies on the effects of *P. mirifica* on osteoblast and osteoclast cells support the results of this study on bone repair in osteoporotic monkeys (Tiyasatkulkovit et al., 2012; Suthon et al., 2016[b]). The administration of *P. mirifica* extract or puerarin increased the mRNA expression level of alkaline phosphatase (*Alp*) and osteoprotegerin (*Opg*) and decreased the receptor activator of nuclear factor kappa-B ligand (*Rankl*)/*Opg* mRNA ratio in rat osteoblast-like UMR 106 cells (Tiyasatkulkovit et al., 2012) and primary baboon osteoblasts (Tiyasatkulkovit et al., 2014). In primary baboon osteoblasts, *P. mirifica* extract also increased collagen Type I mRNA expression (Tiyasatkulkovit et al., 2014). The mechanism of action was shown to initially pass through the estrogen receptor, since the upregulation of *Alp* mRNA levels was abolished by pre-treatment with the estrogen receptor antagonist, ICI182780 (Tiyasatkulkovit et al., 2012). However, the subsequent underlying mechanisms of actions of *P. mirifica* extract or other phytoestrogens on bone healing in monkeys are still unknown and need to be elucidated in the future.

One serious limitation of this study was the number of animals used, especially for the CT scan. It is difficult to acquire postmenopausal monkeys and it is much more difficult to recruit sufficient osteoporotic postmenopausal animals at the same time. So far, naturally osteoporotic monkeys are the most suitable animal models to represent naturally post-menopausal osteoporotic women. Unfortunately, the progress of bone healing has a high variation between individuals, the 3D-CT images of only three monkeys per group could not reach a statistically significant difference. However, the qualitative data from one monkey in the PMP1000 group evidently showed a greater progression of bone healing compared to the PMP0 monkeys. Close examination of the 3D-CT image of this monkey revealed that during the reduction in depth and area of the bone defect, the perimeter actually increased. Combining the CT data and CT image together, we hypothesize that the shallower depth and wider perimeter of the bone defect at month 16 of the PMP1000 treatment was due to the initiation of the remodeling process (bone resorption followed by its formation) at the site of the iliac crest biopsy occurring from the bottom before moving to the edge which has a high bone surface area.

With respect to the bone histology, PMP0 monkeys showed a thick layer of fibrocartilage cells while the PMP1000 monkeys had fewer fibrocartilage cells but a higher level of new bone formation. This indicates that bone healing in the PMP0 individuals was at the early stage of fibrocartilaginous callus formation because the fibrocartilage cells were not yet differentiated into a soft callus, while in the PMP1000 monkeys it was in the late stage of fibrocartilaginous formation up to the early stage of bony callus formation since chondrocytes or osteocytes could be observed together with the existence of woven bone. This leads to the conclusion that bone healing can occur, in general, in the non-treated PMP0 monkeys, but the PMP1000 treatment accelerated the bone healing process.

Even though the progression of bone healing gradually occurred throughout 16 months of the experiment, it should be noted that the bone defect of both the PMP1000 and PMP0 groups was still non-union at month 16 of the study period. Based on the fact that the age of monkeys in this study was over 20 years old, cellular senescence and alteration of the microenvironment surrounding the bone cells might be the cause of the incomplete bone healing. It has been reported that aging affects the proliferation and functional ability of osteal macrophages (Gibon et al., 2016), which are crucial in bone repair for coordinating the crosstalk between osteoclasts and osteoblasts (Cho, 2015). Furthermore, the bone remodeling cycle by osteoblasts and osteoclasts also became more lacking with age (Szulc and Seeman, 2009), potentially due to a reduction in osteoblast precursors, mesenchymal stem cells and osteoblast life span (Boskey and Coleman, 2010). A study in non-human primates also showed a similar trend in that the osteoclast precursor, hematopoietic cells, declining with age (Lee et al., 2005).

In conclusion, PMP treatment could accelerate the progression of bone fracture healing in naturally postmenopausal osteoporotic monkeys, in addition to the previously reported amelioration of bone loss (Kittivanichkul et al., 2016[b]). Therefore, PMP has a high potential to be developed as an anti-osteoporotic agent and also for bone fracture healing for use in humans or pet animals (e.g., dogs) whose cortical bone possesses a Haversian system and whose bone remodeling processes are similar to those in monkeys (Egermann et al., 2005).

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บทคัดย่อ

ศักยภาพของกวาวเครือขาวซึ่งเป็นพืชสมุนไพรที่มีสารไฟโตรเอสโตรเจน ในการประสานเนื้อกระดูกในลิงที่อยู่ในภาวะกระดูกพรุน

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จากที่มีรายงานผลของกวาวเครือขาวต่อโรคกระดูกพรุนในหนูและลิง แต่ไม่มีผลการศึกษาต่อการประสานของกระดูกที่หัก จึงได้คัดเลือกลิงแสมวัยหมดประจำเดือนและกระดูกพรุนมาตัดชิ้นเนื้อกระดูกเชิงกรานออก แบ่งลิงออกเป็นสองกลุ่ม ๆ ละ 5 ตัว คือ กลุ่มที่ได้รับอาหารปกติ (PMP0) และกลุ่มที่ได้รับอาหารผสมผงกวาวเครือขาว 1000 มิลลิกรัม/กิโลกรัม/วัน (PMP1000) นาน 16 เดือน ติดตามการประสานของกระดูกจากภาพถ่ายเอกซเรย์ (X-ray) แล้วสุ่มเลือกลิง 3 ตัว/กลุ่ม มาถ่ายภาพเอกซเรย์คอมพิวเตอร์ (3D-CT) ในเดือนที่ 0 8 และ 16 จากนั้นเลือกลิงที่กระดูกประสานดีที่สุดมาตัดชิ้นเนื้อกระดูกเชิงกรานออกอีกครั้งในเดือนที 16 เพื่อนำไปวิเคราะห์ทางมิญชวิทยา จากภาพ X-ray พบว่าเส้นรอบวงและพื้นที่ที่ตัดชิ้นเนื้อกระดูกออกในลิงกลุ่ม PMP1000 ลดลงเร็วกว่าลิงกลุ่ม PMP0 โดยพบความแตกต่างของเส้นรอบวงระหว่างลิงทั้งสองกลุ่มในเดือนที่ 3, 6 และ 8 จากผล 3D-CT พบว่าลิงกลุ่ม PMP1000 มีการประสานของกระดูกดีกว่าลิงกลุ่ม PMP0 อย่างสอดคล้องกับผลทางมิญชวิทยาที่พบเนื้อกระดูกใหม่เพิ่มขึ้นมากกว่า แต่มีเซลล์กระดูกอ่อนเส้นใยน้อยกว่า การทดลองนี้แสดงให้เห็นว่านอกจากผลต่อโรคกระดูกพรุนแล้ว กวาวเครือขาวยังช่วยกระตุ้นการประสานของกระดูกที่หักด้วย

คำสำคัญ: มวลกระดูก ภาวะพร่องฮอร์โมนเอสโทรเจน ลิงแสม กระดูกพรุน กวาวเครือขาว เอกซเรย์

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