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ORIGINAL PAPER

Homeopathic *Symphytum officinale* increases removal torque and radiographic bone density around titanium implants in rats

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Introduction: This study evaluated the effect of _Symphytum officinale_ in homeopathic potency (6cH), on the removal torque and radiographic bone density around titanium implants, inserted in rats tibiae.
Methods: Implants were placed in male rat tibiae, and the animals randomized to two groups (Control and _S. officinale_ 6cH treated), which were evaluated at 7, 14, 28 and 56 days post-implantation. Radiographic bone density was measured at 6 points around the implant, using digital radiographic images, when implants were inserted and at sacrifice. Removal torque of the implants was also evaluated.
Results: Both removal torque and radiographic bone density evaluation showed that _S. officinale_ 6cH treatment enhanced bone formation around the micro-implants, mainly at 14 days. At 56 days, the radiographic bone density was higher in the treated group.
Conclusions: We conclude that _S. officinale_ 6cH enhances, principally at the early stages of osseointegration, bone formation around titanium implants in rats' tibiae, based on radiographic and mechanical analysis. *Homeopathy* (2010) **99**, 249–254.

Keywords: Radiography; Bone density; Torque; *Symphytum officinale*; Osseointegration; Titanium implants; Experimental; Homeopathy

Introduction

Symphytum officinale (*Boraginaceae*), is a common plant that has been used as herbal medicine. It was used by Roman soldiers to treat combat bone fractures 2000 years ago. *S. officinale* leaves and roots are currently used by some physicians to treat bone fractures, tendon damage, joint disease and ulcerations in the gastro-intestinal tract.[1,2] In addition, *S. officinale* can be applied externally in the treatment of ankle, joint subluxation and osteoarthritis of the knee.[3] Several clinical trials have demonstrated its efficacy.[1,3–5] *S. officinale* contains Allantoin, carotene, hydroxycinamon acid derivatives, essential oils, vitamin B12 and zinc, which may be responsible for its healing properties. However, the presence of hepatotoxic pyrrolizidine alkaloids (PAs) in *S. officinale* restricts its consumption for long periods.[6,7]

S. officinale is recommended in the homeopathic literature to accelerate bone healing, reducing the time to consolidate bone fractures and accelerate bone mineralization. Homeopathic solutions are extremely diluted and thus likely to be less noxious to the organism.[8]

In order to achieve predictable osseointegration between bone and implant, it is necessary to respect some basic

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treatment principles such as minimal surgical trauma, absence of infection, biocompatible hardware and adequate time without disturbance.[9,10] Efforts are being made to reduce the time for bone healing, such as improvement of implant surface and immediate loading implants.[11–15] However, the immediate loading technique is indicated only in patients where a substantial amount of bone is available. Patients who have bone loss, poor bone quality and occlusion dysfunctions have been treated in accordance with the conventional implant treatment protocol, which means a longer bone healing period and this is a disadvantage of implant treatment. New approaches that could enhance healing time around dental implants are needed.

The present study evaluated the influence of the *S. officinale*, in a homeopathic dilution (6cH), on the removal torque and radiographic bone density around titanium implants, inserted in rats' tibiae.

Material and methods

The study protocol was approved by the Ethics in Animal Research Committee of the School of Dentistry of Araraquara (UNESP, Brazil), and it is in compliance with the applicable ethical guidelines and regulations of the International Guiding Principles for Biomedical Research involving animals (Geneva, 1985).

Animals

64 *Rattus norvegicus* rats were used (*Holtzmann, albinus*, male, adults, and weighing around 220 g). The rats were kept at a special facility at São Paulo State University – UNESP, School of Dentistry of Araraquara, in individual cages and in a room with a 12 h light/dark cycle and temperature between 22 and 24°C. They were fed a standard laboratory diet and given tap water.

After a 15-day acclimatization period, the animals were submitted to implant surgery and randomly assigned to one of two experimental groups: 32 animals administered *S. officinale* 6cH (Group S) and 32 Control animals (Group C). Each group of animals was randomly divided into four sub-groups for euthanasia at the 7th, 14th, 28th or 56th post-operative days, in a total amount of 8 animals/group/period of observation.

Homeopathic treatment

The manufacturing of homeopathic remedies is based on the processes of dilution and succussion (vigorous shaking), called potentization.[16–20]

In this study, the homeopathic dilution concentration used was 6cH, in water. *S. officinale* mother tincture (Farmácia Reativa, Araraquara, São Paulo, Brazil), obtained from roots and leaves, and in a 40% alcoholic basis, was serially diluted. The botanical characterization of the used plants, both morphological and chemical, was performed by the manufacturer of the tincture. To prepare the 6cH concentration, one part of the mother tincture was diluted into 99 parts of alcohol, six successive times, and mechanically succussed 100 times, according to Brazilian Homeopathic Pharmacopoeia method.[8,21]

Ten drops of *S. officinale* 6cH daily were mixed with 40 ml of water, all of which was drunk by the animals every day, until sacrifice. After the animals had consumed all the medicated water, they were given tap water again. They drank 40–100 ml of water per day. The control group received only water, the vehicle used for the drug.

Implant surgery

Animals were anesthetized by intramuscular injection of xylazine (6 mg/kg body weight, Francotar, Virbac do Brasil Ind. Com. Ltda., São Paulo, Brazil) and ketamine (70 mg/kg body weight, Vyrbaxil, Virbac do Brasil Ind. Com. Ltda., São Paulo, Brazil). An incision, approximately 20 mm long, was made on the medial side of the right and left tibia proximal metaphysis. Bone tissue was carefully exposed, and bicortical implant beds, one in each tibia, were prepared using a progressive sequence of cooled rotary drills. Cooling solution used was sterile saline.

A micro-implant – 4.0 mm in length and 2.2 mm in diameter, with machined surface (AS Technology, São José dos Campos, SP, Brazil) was inserted. All implants were then covered by a collagen membrane (Genius-Baumer, São Paulo, Brazil), with rounded edges and hydrated with sterile saline solution. The soft tissues were then repositioned and sutured to achieve primary closure (Vycril 4.0, Ethicon, São Paulo, SP, Brazil). Each animal received an intramuscular injection of 24,000 IU penicillin G-benzathine (Pentabiótico Veterinário Pequeno Porte, Fort Dodges Saúde Animal Ltd., Campinas, Brazil) post-surgically.

Radiographic bone density – image acquisition and analysis

For the radiographic evaluation, of the left tibia, two digital radiographs of the implants were taken, one immediately after surgery (initial radiography), and the second at sacrifice after 7, 14, 28 or 56 days (final radiography). Complementary metal-oxide semiconductor (CMOS) equipment (Schick Technologies Inc., Dialom Dental Products, Long Island City, NY) was used. The tibiae were fixed in a holding device with the vertical long axis of the implant positioned perpendicularly to the central X-ray beam and parallel to the sensor at 40-cm focus-object distance. The X-ray unit was operated at 70 KVp, 10 mA, and 0.2 s (Expectro 70x, Dabi Atlante, Ribeirão Preto, SP, Brazil). Image resolution was 635 ppi (pixels per inch), the size of the image was 900 × 641 dpi and pixel size was 40 μm. Images were stored in the TIFF (Tagged Image File Format) without compression (8 bits with 600 dpi resolution).

The radiographic bone density in the defects was determined by the analysis of the gray scale in an area of 5 × 5 pixels, in regions denominated superior cortical, cancellous, and inferior cortical, on both sides of the implant (Figure 1), as the regions of interest (ROIs). This analysis was done using the image-analysis software Image Tool 2.03 (UTHSCA, San Antonio, Texas, EUA), which provided the gray scale

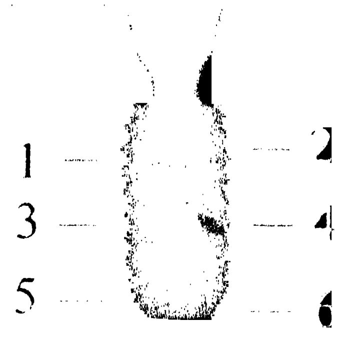

Figure 1 ROIs for analysis of radiographic bone density (1 and 2 – superior cortical; 3 and 4 – cancellous; 5 and 6 – inferior cortical).

average and standard deviation in these pre-determined regions by means of a histogram graph. The bone density calculations were performed by the average gray levels of the evaluated ROIs, which were divided by the gray level of the implant, to compensate minimal differences among radiographs, since the density of the metallic standard was the same in all specimens.[22,23]

Removal torque evaluation

Analysis of the removal torque was made after the final radiograph, taken after animal sacrifice. Prior to sacrifice, one of the authors (CES) randomized them. The authors making the evaluation (MMB and RSN) were 'blind' to treatment. The right tibia was dissected to expose the implant, allowing the attachment of a torque meter with a scale range of 0.1–10 N cm and divisions of 0.05 N cm (Tohnichi, Shangai, China). A wrench was attached to the implant head to apply torque in the reverse direction of implant placement, until complete rupture of the bone/implant interface was signaled by rotation of the implant. This value was considered as the torque necessary for the breakdown of osseointegration.[24,25]

Biochemical evaluation

For the animals of the 56-day groups only, serum Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated to evaluate hepatic function and or enhanced production of bone related enzymes such as ALP.

Statistical analysis

The Shapiro–Wilk test was used to verify data distribution. Data from radiographic bone density, removal torque and AST, ALT and ALP values were analyzed statistically by two-tailed *t* test. Significance level was set at 5%. Comparisons between the different groups and periods were made.

Results

All analyzed data were normally distributed. The removal torque analysis showed a statistically significant difference when comparing groups Control (0.57 ± 0.10 N cm) and *Symphytum* (0.76 ± 0.09 N cm) at 14 days ($p < 0.001$, analysis of variance – ANOVA), no significant difference was found between the groups at 7 days (0.59 ± 0.14 and 0.70 ± 0.23 N cm, respectively), 28 (0.74 ± 0.13 and 0.66 ± 0.15 N cm, respectively) or 56 days (0.77 ± 0.18 and 0.63 ± 0.05 N cm, respectively), and multiple intra-group comparison did not showed significant results (Figure 2).

Data from the radiographic analysis of the ROIs are given in Figure 3a–c and Table 1. *Symphytum* groups had statistically lower radiographic bone density values, compared to initial and the final images, at 7 days. However, at 14 and 56 days *Symphytum* groups had radiographic bone density values statistically higher, compared to control values. Considering only the final images values, comparing both groups, results showed that the values for the *Symphytum* group, at 14 days, were statistically higher when compared to Control, in all analyzed regions. With exception of the superior cortical region, the same was observed at 56 days.

Biochemical analysis (Figure 4) showed that ALP values were statistically increased in the *Symphytum* group (72.20 ± 15.21 UI/ml) compared to control (19.08 ± 3.47 UI/ml). There were no significant differences in ALT or AST.

Discussion

Several surveys in Europe and the USA have demonstrated a rise of botanical medicine use in the last few years, and this is probably based on the popular belief that these treatments are natural, harmless, and a desire for wellness and quality of life. Access to these kinds of therapy is unrestricted in most countries and relatively cheap.[26]

Homeopathic treatment is based on internal cure, where the organism is stimulated to cure itself.[27] Recent studies on the biological and clinical effects of homeopathic substances suggest that the information is transmitted to the cells by the potentised solution, even if the quantity of the

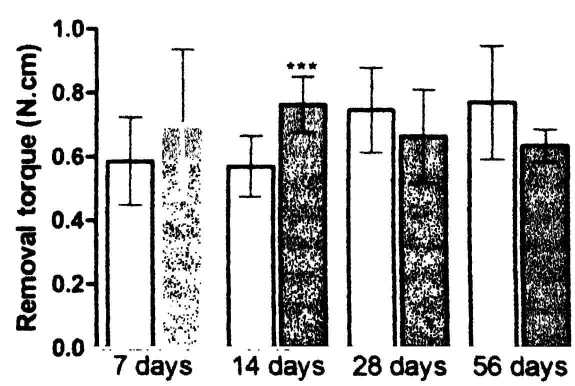

Figure 2 Average and standard deviation values for removal torque. ***$p < 0.001$ – *t* test (comparison between the two groups in each period of evaluation).

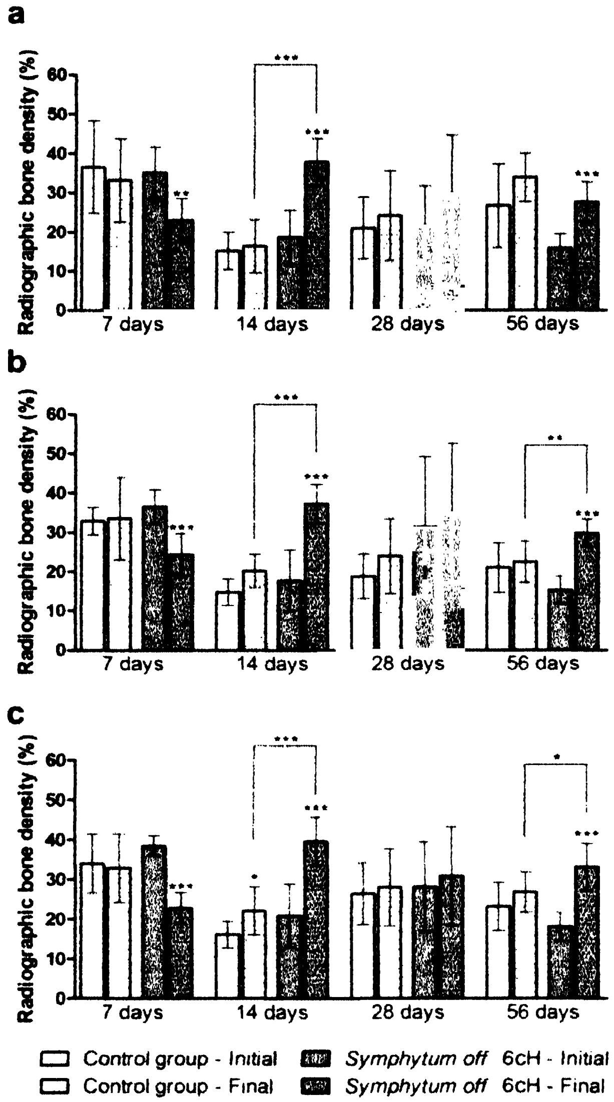

Figure 3 Radiographic bone density averages and standard deviation, in the superior cortical (a), cancellous (b) and inferior cortical (c) regions. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ – t test.

active drug is minimal the literature is very clear in encouraging further repetitions of published studies using high dilutions, in an attempt to elucidate the phenomenological features involved in these studies and prove their repeatability of results, to create reliable models for testing drugs in such low concentrations.[28,29]

Animals are an interesting way of studying homeopathic treatments because there is no possibility of placebo effects.[30]

The establishment of the validity of a homeopathic method requires that it adhere to the same standards as non-homeopathic methods, that the research should be blinded, evaluate the placebo effect, and the relative magnitude of any effect,[31] as we did in this paper.

In the present study, the *S. officinale* seems to stimulate bone repair at 14 days. This was reflected in the acceleration of the regeneration and maturation in periimplant bone tissue. This may have been largely completed by 28 days, so that after 14 days further changes in bone tissue

Table 1 Radiographic bone density averages and standard deviation, in the superior cortical, cancellous and inferior cortical regions (%)

Group	*Region*	*7 days*		*14 days*		*28 days*		*56 days*	
		Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	*Initial*	*Final*
Control	Superior cortical	36.55 ± 11.75	33.22 ± 10.50	15.25 ± 4.70	16.44 ± 6.75	21.00 ± 7.92	24.15 ± 11.46	26.60 ± 10.63	33.84 ± 6.20
	Cancellous	32.87 ± 3.42	33.62 ± 10.48	14.83 ± 3.39	20.22 ± 4.21	18.80 ± 5.67	23.96 ± 9.47	21.02 ± 6.32	22.45 ± 5.32
	Inferior cortical	33.98 ± 7.43	32.95 ± 8.64	16.17 ± 3.34	22.11 ± 6.02	26.40 ± 7.82	28.02 ± 9.74	23.21 ± 6.12	26.82 ± 5.08
Symphytum officinale	Superior cortical	35.18 ± 6.53	23.10 ± 5.45	18.68 ± 6.90	37.92 ± 5.93	21.68 ± 10.01	29.74 ± 14.80	15.80 ± 3.69	27.59 ± 5.09
	Cancellous	36.57 ± 4.36	24.32 ± 5.52	17.77 ± 7.80	37.31 ± 4.92	31.42 ± 17.72	34.83 ± 17.82	15.28 ± 3.52	29.82 ± 3.49
	Inferior cortical	38.52 ± 2.55	22.80 ± 3.94	20.91 ± 7.95	39.60 ± 6.05	28.18 ± 11.23	30.86 ± 12.41	18.08 ± 3.62	33.10 ± 5.90

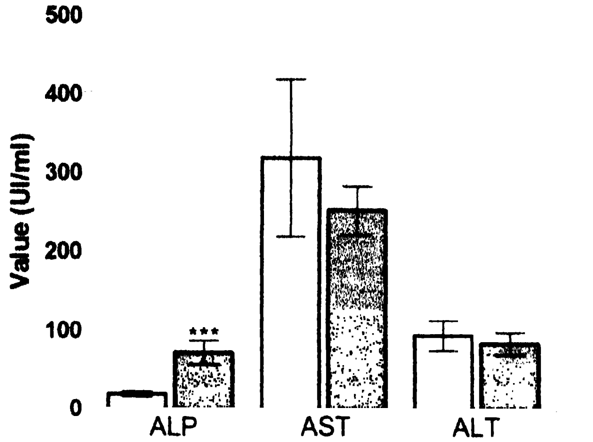

Figure 4 Averages and standard deviation of the sera evaluation for ALP, AST and ALT. ***$p < 0.001$ – t test (comparison between the two groups, in the 56 days period of evaluation).

could not be detected radiographically. The initial loss of bone density in the *Symphytum* group at 7 days is puzzling, but could be explained by initial necrosis that follows bone injuries, such as drilling for the implant placement, rather than treatment.[10–14,23,32]

Removal torque analysis biomechanically measures the degree of bone to implant contact, known as osseointegration.[32–34] The results for removal torque showed a statistically significant increase in both groups at 14 days. The between-group comparison at 14, 28 and 56 days shows that the average values are similar.

The acceleration in the rate of bone maturation may be explained by the tissue healing properties of *S. officinale*, this effect is attributed mainly to one of its constituents, Allantoin, an anti-inflammatory, analgesic, granulation tissue promoting and anti-exudative substance. *S. officinale* has a 0.8% content of Allantoin.[35] This component has special affinity to the periosteum and bone, it has also been reported to benefit skin and mucosa because of its analgesic effects and fibroblastic stimulation.[36–38] This was one of the major reasons why *S. officinale* was chosen for this research.

The main problem is that *S. officinale* contains hepatotoxic PAs, such as lasiocarpine, symphytine and their related N-oxides.[35,39] These alkaloids are hepatotoxic in both animals and humans.[40] The main liver injury is veno-occlusive disease leading to cirrhosis and liver failure. The mechanisms by which *S. officinale* causes toxicity are not fully understood, but are related to biotransformation and metabolic activation of enzymes in the liver.[39] Hepatic transamidases process the aminoacids in liver, raised levels of these enzymes, mainly AST and ALT suggest impaired liver function.[40]

In our study ALP was statistically higher in the S than in the C group. This enzyme has three different isoforms – bone, kidney and liver and the bone isoform is an early marker of bone maturation during mineralization.[41] However, in our study we measured the non-specific tissue isoform of ALP, and this was the only enzyme that increased with *S. officinale* therapy. This could be related to the acceleration of the rate of bone maturation. The fact that there was no change in the specifically liver enzymes ALT and AST suggests that the elevated ALP was not of liver origin.

Our biochemical results do not show any impairment of the hepatic function allied to the homeopathic treatment, suggesting that highly diluted homeopathic *S. officinale* is not hepatotoxic.[6,35,40]

Conclusion

Considering these results and considering on the limitations of the model that was used, we conclude that *S. officinale* 6cH may enhance, principally at the early stages of osseointegration, bone formation around titanium implants placed in rats' tibiae, without causing biochemically detectable liver damage.

Conflict of interest

Authors declare that there were no conflict of interest associated to this publication.

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