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Original Article

Effect of Bushen Guchi Pills on alveolar bone reconstruction in rats with periodontitis

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Abstract: Objective: This study was designed to analyze the effects of Bushen Guchi Pills on remodeling the alveolar bone of rats with paradentitis. Methods: Thirty healthy adult SD rats were included and randomized into three groups. Two groups were processed as models of rats with paradentitis, and further divided into the model group, subject to no treatment and the treatment group, with Bushen Guchi Pills. The 3rd group was the control group consisting of normal rats without any treatment. The three groups were compared for alveolar bone remodeling, serum bone turnover markers and Micro-CT examination results. Results: One month after treatment, an ascending order was established as the treatment group, the model group and the normal group for osteocalcin (OCN) (P<0.05), the treatment group, the normal group and the model group in bone volume/total volume (BV/TV) (P<0.05), the model group, the treatment group and the normal group for trabecular number (Tb.N) (P<0.05), the normal group, the treatment group and the model group for pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), trabecular separation (Tb.Sp) and alveolar bone absorption (P<0.05). Conclusion: Bushen Guchi Pills have demonstrated its treatment values in promoting alveolar bone remodeling in rats with paradentitis by significantly improving alveolar bone metabolism.

Keywords: Paradentitis, rats, Bushen Guchi Pills, treatment, alveolar bone

Introduction

Paradentitis is a stomatological chronic infectious disease mainly found in the supporting tissues of the teeth. It affects patients in eating food and prominently compromises their health and quality of life [1]. Cobb CM [2] pointed out in his study that bacterial infection is the primary cause of paradentitis, during which, the microbes interact with the host and affect the development and progress of paradentitis. Once the microbes within the host are out of balance, the periodontium will be damaged and diseases will occur.

Currently, more and more in-depth studies on the treatment of paradentitis with traditional Chinese methods are being carried out. According to traditional Chinese medicine, internal factors, i.e., kidney deficiency, and external factors, i.e., damp heat [3], contribute to paradentitis. The treatment of paradentitis will focus on replenishing kidney Qi and improving kidney deficiency to strengthen teeth [4]. As a traditional Chinese medicine (TCM) preparation based on a traditional kidney tonifying prescription in China, the Pills of Six Drugs with Rehmannia, Bushen Guchi Pills take advantage of Drynaria Rhizome to improve dentary damage, Radix Achyranthis Bidentatae to strengthen tendons and bones, Placenta Hominis to greatly tonify the original Qi, Matrimony vine to tonify the kidneys and promote the secretion of saliva, Rhizoma Alismatis, *Chrysanthemum indicum*, *Poria cocos* and Radix Rhapontici seu Radix Echinopsis to clear damp heat and eliminate fire toxin, thus achieving the ultimate goals of reinforcing healthy Qi to eliminate pathogenic factors and strengthening teeth [5, 6].

However, so far, most clinical studies on paradentitis have paid more attention to western

solutions, and less consideration to the traditional Chinese methods combined with animal experiment. In the present study, 30 rats were used to establish the model of periodontitis, in order to specifically explore the application value of Bushen Guchi Pills in TCM with the expectations of finding more feasible methods for clinical treatment of paradentitis.

Materials and methods

Materials

Thirty (15 females and 15 males) healthy and qualified adult SD rats with mean body mass of about 200 g were selected, and randomized into three groups, the normal group, the model group and the treatment group. The rats were kept in a space with normal sun exposure, moderate temperature between 18 and 25°C, humidity from 60% to 80%, and sufficient food and water, and were managed in strict accordance with the provisions of Animal Protection Association.

Methods

Apparatuses and equipment: micro-CT (Mi-cro-CT 80), optical microscope, needle holder, anaerobic bacteria culture incubator, wiring clip, flat tip forceps, eye scissors, lavage equipment, and bone forceps.

Modeling: there were 10 rats kept normally, the other 20 rats were modeled with the methods of binding the left first molar with orthodontic wire, and feeding the rats water containing 20 mg/d amoxicillin (125 mg/capsule) for consecutive 3 days in order to suppress the endogenous bacteria which are not advantageous to the growth of porphyromonas gingivalises. The next step was to inoculate the gingival sulcus of the first molar on the left upper mandible with 1 mL suspension of porphyromonas gingivalis (1.5×10^{12} CFU/L) 3 times at an interval of 2 days. Those modeled rats were fed with 10% high-carbonhydrate drinking water. The whole modeling process lasted 3 weeks from the completion of wire binding on the first molar of the upper mandible, after which, 2 rats were selected from each of the group and sacrificed to separate the upper mandibles which were then fixed in 10% neutral buffered formalin for 24 h, and pathologically sliced to observe the changes in the periodontium of the first molar of the upper mandible and judge the modeling results.

Treatment: the remaining 8 rats were selected for intragastric administration with 10 mL/(kg·d) normal saline from the normal group; and the also for model group where 8 rats were selected from the treatment group with intragastric administration with 0.8 g/(kg·d) Bushen Guchi Pills (specification: 4 g*8 bags, approval document No.: GYZZ Z51021248, manufacturer: Chengdu Jiuzhitang Jinding Pharmaceutical Co., Ltd.) dissolved in water. All three groups were continuously treated for 1 month.

Observation indices

(1) Serum bone turnover markers [7]: after modeling and 1 month of continuous treatment, the three groups were prohibited from water and food for 12 h. Plasma was then collected from their arteria cruralis, centrifuged at 3000 rpm for 10 min. The serum was collected into EP tubes and stored under -8°C. Radioimmunoassay (RIA) was used to detect the levels of osteocalcin (OCN) and pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP).

(2) Alveolar bone: alveolar bone was assessed by Micro-MT, a 3 d imaging examination. The Micro-MT worked 1,000 times based on the parameters of pixel of 2048*2048, layer distance of 10 μm, integration time of 300 ms and interval of 180°. The left alveoli from the upper mandible of the three groups were preserved in 10% neutral formalin, and then were taken out for image collection on a Micro-CT where each sample was scanned for about 85 min. Afterward, the alveolar bone in the branching area of the 1st molar root was selected as the region of interest (ROI) for analysis based on 64-digit collection/reconstruction/analysis software, and for 3D modeling to obtain data. Indices to be tested include bone volume/total volume (BV/TV), trabecular number (Tb.N), and trabecular separation (Tb.Sp). Amongst them, BV/TV is the ratio between the volume of bony structure and the total volume of the sample [8], and a larger ratio indicates greater trabecular content. Tb.N is a descriptive indicator of bone mass change. With a fixed trabecular thickness, Tb.N interacts with bone mass positively [9]. Tb.Sp is a descriptive indicator of trabecular structure, which increases to reflect the more significant separation [10].

Table 1. Comparison amongst the three groups for serum bone turnover markers after modeling ($\bar{x}$ ± s, ng/mL)

Group	n	OCN	ICTP
Treatment group	10	1.6243±0.2597*	2.4084±0.4593*
Model group	10	1.6589±0.2621*	2.4028±0.4705*
Normal group	10	0.5928±0.1241	1.3383±0.3872

Note: *P<0.05 as compared with the normal group.

Table 2. Comparison amongst the three groups for serum bone turnover markers after 1-month treatment ($\bar{x}$ ± s, ng/mL)

Group	n	OCN	ICTP
Treatment group	10	0.5043±0.1089[&]	2.0451±0.4121[&]
Model group	10	0.8621±0.1572[&,*]	3.0561±0.4612[&,*]
Normal group	10	1.8062±0.2537	1.4645±0.4035

Note: [&]P<0.05 as compared with the normal group, and [*]P<0.05 as compared with the treatment group.

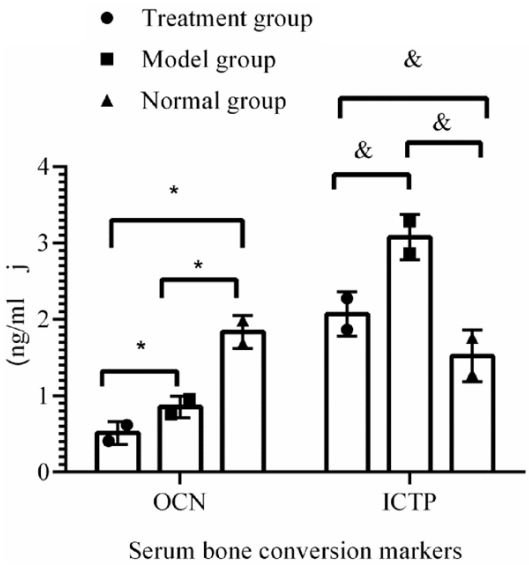

Figure 1. Comparison amongst the three groups for serum bone turnover markers. For OCN, the model group was higher than the normal group (P<0.05), and the normal group was higher than the treatment group (P<0.05); for ICTP, the model group was higher than the treatment group (P<0.05), and the treatment group was higher than the normal group (P<0.05); for comparison between every 2 groups, * represents P<0.05 for OCN, and & represents P<0.05 for ICTP.

(3) Alveolar bone absorption: after modeling and treatment, left alveolar bones were collected from the three groups as the testing samples to observe the alveolar bone absorption after thoroughly removing the soft tissue to reserve the hard tissue only. Under a microscope, each sample was measured for the distance from the enamelo-cemental junction of the first molar to the alveolar ridge top, and each tooth position was measured at the cheek, the near, middle and far points of lingual side, whose sum constitutes the alveolar bone absorption of the tooth [11].

Statistical analysis

Statistical analysis was performed with SPSS22.0. In case of numerical data it was expressed as Mean ± Standard Deviation, independent-sample T test was used for group comparisons. For all statistical comparisons, P<0.05 was considered as statistically significant.

Results

Comparison amongst the three groups for serum bone turnover markers after modeling

After modeling, OCN and ICTP levels in the treatment group and the model group were higher than those in the normal group (P<0.05), while no statistical difference was observed between the treatment and the model groups (P>0.05, **Table 1**).

Comparison amongst the three groups for serum bone turnover markers one month after treatment

One month after treatment, OCN level in treatment group was lower than that in model and normal groups, and OCN in model group was lower than that in normal group (P<0.05). While ICTP level in model group was higher than that in the treatment and normal groups, and ICTP in the treatment group was higher than that in the normal group (P<0.05, **Table 2**; **Figure 1**).

Comparison amongst the three groups for micro-CT examination results of alveolar bone one month after treatment

One month after treatment, BV/TV in the model group was lower than that in the treatment and normal groups, while that in the normal group was higher than that in the treatment group (P<0.05). One month after treatment, Tb.N in the normal group was higher than that in the treatment and model groups, while that in the

Table 3. Comparison amongst the three groups for micro-CT examination results of alveolar bone after 1-month treatment ($\bar{x} \pm s$)

Group	n	BV/TV (%)	Tb.N (mm^{-1})	Tb.Sp (mm)
Treatment group	8	0.3042±0.0101*	3.1245±0.1001*	0.5752±0.0201*
Model group	8	0.1817±0.0201*,&	2.0890±0.2001*,&	0.6111±0.0301*,&
Normal group	8	0.4642±0.0151	5.8660±0.1001	0.1308±0.0110

Note: *P<0.05 as compared with the normal group, and &P<0.05 as compared with the treatment group.

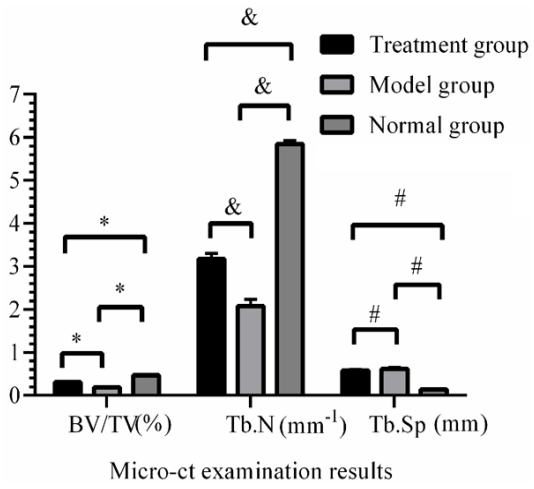

Figure 2. Comparison amongst the three groups for micro-CT examination results of alveolar bone after 1-month treatment. For BV/TV and Tb.N, the normal group was higher than the treatment group (P<0.05), and the treatment group was higher than the model group (P<0.05); for Tb.Sp, the normal group was lower than the treatment group (P<0.05), and the treatment group was lower than the model group (P<0.05). *, & and # represents P<0.05 for BV/TV, Tb.N and Tb.Sp respectively as compared between every 2 groups.

treatment group was higher than that in the model group (P<0.05). One month after treatment, Tb.Sp in the model group was higher than that in the normal and treatment groups, while that in the treatment group was higher than that in the normal group (P<0.05, **Table 3**; **Figures 2** and **3**).

Comparison amongst the three groups for alveolar bone absorption after modeling

After modeling, the alveolar bone absorption in the treatment and model groups was higher than that in the normal group (P<0.05), while after treatment, no statistical difference was observed between the treatment group and the model group in alveolar bone absorption (P> 0.05, **Table 4**).

Comparison amongst the three groups for alveolar bone absorption one month after treatment

One month after treatment, the alveolar bone absorption in the model group was higher than that in the treatment and normal groups, of which that in the treatment group was higher than that in the normal group (P<0.05, **Table 5** and **Figure 4**).

Discussion

In TCM, paradentitis falls into the category of "gingival atrophy" and "gringival hemorrhage", and its occurrence is attributed to deficiency of kidney Qi, intense stomach fire, and insufficiency of vital energy and blood. All bones and teeth in our body are nourished by kidney Qi, and once the body is deficient of kidney essence, teeth may become lose and fall out [3]. Based on such a theory, the TCM treatment of paradentitis focuses on tonify Qi of the kidney, clearing away heat and toxic material [12]. In the present study, the TCM preparation Bushen Guchi Pills are made of various traditional Chinese medicines, including *Astragalus mongholicus* and Chinese yam which support healthy energy, strengthens the spleen and replenish Qi while placenta hominis, Radix Rehmanniae Recens and Radix Rehmanniae Praeparata nourish kidney Qi, tonify essence and supplement marrow; Radix Achyranthis Bidentatae, *Poria cocos* and Rhizoma Alismatis conduct fire downward and eliminate dampness and dieresis; the root of red-rooted salvia, *Chrysanthemum indicum*, Radix Rhapontici seu Radix Echinopsis, Radix Curcumae, and the root bark of the peony tree clear away heat and toxic material, cool the blood and reduce swelling; *Cinnamomum cassia* seeks Yang in Yin and relieves fire of deficiency type; *Schisandra chinensis*, *Drynaria rhizome*, lignum millettiae and *Matrimony vine* strengthen the bones and muscles, and tonify the liver and kidney [13, 14].

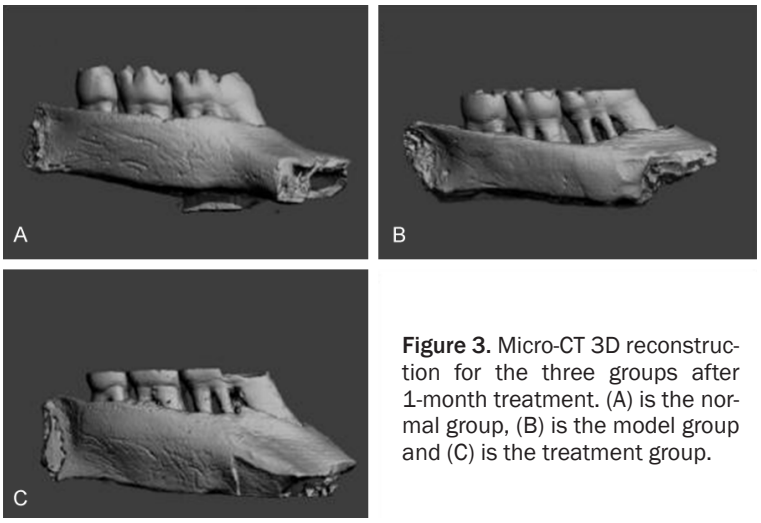

Figure 3. Micro-CT 3D reconstruction for the three groups after 1-month treatment. (A) is the normal group, (B) is the model group and (C) is the treatment group.

Table 4. Comparison amongst the three groups for alveolar bone absorption after modeling ($\bar{x}$ ± s, μmL)

Group	n	Alveolar bone absorption
Treatment group	10	7175.62±1015.23*
Model group	10	7095.23±1052.47*
Normal group	10	2935.45±478.51

Note: *P<0.05 as compared with the normal group.

Table 5. Comparison amongst the three groups for alveolar bone absorption after 1-month treatment ($\bar{x}$ ± s, μm)

Group	n	Alveolar bone absorption
Treatment group	8	5748.15±687.92*
Model group	8	7516.49±1105.37
Normal group	8	3728.94±563.27*,&

Note: *P<0.05 as compared with the model group, and &P<0.05 as compared with the treatment group.

Those medicinal materials can give full play to the effect of clearing away heat, activating blood circulation, and tonifying the kidney and Qi.

Wen Q et al. [15] applied Bushen Guchi Pills to study the treatment effects on patients with paradentitis, and the results revealed that, after 3-months of treatment, the accumulated points of syndromes, probing depth (PD), plaque index (PLI) and attachment loss (AL) were reduced, and the conditions of patients with paradentitis were improved. Zhi-Guo A N et al. [16] pointed out in their study that the application of Bushen Guchi Pills in the auxiliary treatment of various types of paradentitis can clearly ameliorate the PD of periodoatal pus pocket, bleeding index, and gingival index (GI); while Zhou W et al. [17] found in their study that Bushen Guchi Pills can reinforce the chemotaxis and phagocytosis of neutrophile granulocyte in patients with invasive paradentitis. The study of Rattanasompattikul M et al. [18] indicated that Bushen Guchi Pills played a role contributing to the stability of healthy subgum flora and effective slowing down of reproduction of pathogenic flora. In the present study, OCN and ICTP in the treatment group were significantly improved after 1 month of treatment, and Micro-CT examination of alveolar bone showed significant improvement in BV/TV, Tb.N and Tb.Sp. In addition, alveolar bone absorption was reduced significantly, indicating the good application value of Bushen Guchi Pills in this treatment. The Bushen Guchi Pills are a traditional Chinese medicine prescription based on the Pills of Six Drugs with Rehmannia, and are made by adding the components of Drynaria Rhizome and chrysanthemum. It can improve the host's immune function and promote the restoration and reconstruction of alveolar bone through the combined action of different herbs, which plays a good therapeutic effect on periodontitis. Bushen Guchi Pills can improve the function of adrenal cortex, regulate the activities of some oxidoreductases and ALPs in periodontal tissues, improve the host immune response, increase the level of superoxide dismutase activity, thereby inhibiting lipid peroxidation of the tissues and protecting specific and nonspecific immunologic functions of the body.

Clinical treatment of periodontosis requires remodeling and recovery of alveolar bone. However, changes in alveolar bone in previous treatments were evaluated based on measured serum osteocalcin, alkaline phosphatase, calcium and phosphorus without satisfactory sensitivity and specificity [19]. The measurement indices in the study were ICTP and OCN, of which, ICTP was a highly specific and stable substance obtained by decomposing type I collagen, which can't be further decomposed in

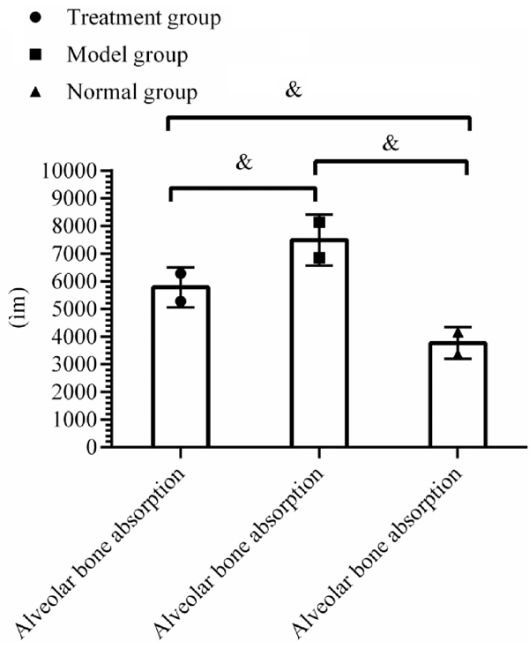

Figure 4. Comparison amongst the three groups for alveolar bone absorption after 1-month treatment. For alveolar bone absorption, the normal group was lower than the treatment group (P<0.05), and the treatment group was lower than the model group (P<0.05). & indicates P<0.05 as compared between every 2 groups.

the kidney [20]. Mishra D et al. [21] discovered a close relationship between ICTP, a typical bone absorption marker, and the histomorphology of bone absorption. Moreover, OCN, a bone turnover marker [22], has played an outstanding role in the regulation of bone calcium metabolism. In the development and progression of paradentitis, bone turnover continues in alveolar bone, and the bone absorption is coupled with bone formation. The present study intervened in the three groups for one month, and it was found that, after 1-month treatment with Bushen Guchi Pills, the rats had reduced ICTP and OCN as compared with the model group, indicating that Bushen Guchi Pills can decrease the alveolar bone conversion in patients with paradentitis, and slow down the absorption of alveolar bone in the progress of paradentitis. Bushen Guchi Pills can effectively regulate bone metabolism, cytokines and endocrine factors in patients with periodontitis, accelerate the bone contact rate of the jaw, and play an important role in improving the condition of OCN.

So far, clinical judgment of alveolar bone reconstruction is based on X-ray and histopathological examination. X-ray imaging only applies to alveolar bone absorption and skeletal density change through two-dimensional observation, which results in limitations in evaluation [23], while histopathological examination covers the inflammatory cell infiltration, alveolar ridge morphology and height in periodontium to judge the damage of paradentitis. However, histopathological examination involves quite complicated slice preparation process and doesn't support quantitative analysis [24]. In the present study, Micro-CT, a highly effective and economic method, was selected to analyze the morphological structure of bone trabecula three-dimensionally. In such an examination, subsequent histologic analysis was not affected though samples were not specially processed, and in the meantime, it supplements histologic analysis to ensure accurate and quantitative evaluation of alveolar bone reconstruction. After treatment for 1 month, the model group was lower than the treatment group and the treatment group was lower than the normal group in BV/TV, while the normal group was higher than the treatment group and the model group, and the model group was lower than the treatment group in Tb.N, and the model group was higher than the treatment group and the normal group, and the treatment group was higher than the normal group in Tb.Sp (P<0.05). In addition, after 1-month treatment, the treatment group was lower than the model group in alveolar bone absorption (P<0.05), indicating that, being treated with Bushen Guchi Pills, rats with paradentitis were improved in alveolar bone conditions, promoted in alveolar bone absorption and accelerated in alveolar bone reconstruction.

In conclusion, the application value of Bushen Guchi Pills in rats with paradentitis includes promoting reconstruction, improving metabolism and assisting transformation of alveolar bone. As a study based on animals, it fails to establish the administration dose of Bushen Guchi Pills, and its possibility of achieving the same effects in a human body. Furthermore, without an extensive coverage and a focus on the administration safety, the present study is limited to the analysis of effects of treatment on alveolar bone. Those shortcomings shall be made up in the future studies to comprehensively explore the application value of Bushen Guchi Pills in the treatment of paradentitis.

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Disclosure of conflict of interest

None.

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