The Effects of Exenatide Microsphere on Serum BGP and ALP Levels in ZDF Rats after Implantation

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ABSTRACT

Purpose: The aim of this project is to investigate the impact of diabetes mellitus and different glycemic control times on early osseointegration of dental implants by expression and significance of serum bone Gla protein (BGP) and alkaline phosphatase (ALP) levels in Zucker diabetic fatty (ZDF) rats after implantation.

Materials and Methods: The animals were divided into three groups, each group with 11 rats and each rat with two dental implants (33 rats and 66 implants in total): group A, diabetic rats with dental implants (controls); group B, diabetic rats treated with insulin and implants placed simultaneously; and group C, diabetic rats treated with insulin until serum glucose at a constant level and then implants be placed. Levels of BGP and ALP in the serum were measured by enzyme-linked immunosorbent assay in each group. The software program SPSS (version 17.0 for Windows) was used to analyze all data; $p < .05$ was set as the level of statistical significance.

Results: At the 7th day, serum levels of BGP in group B and C were lower than that in group A ($p > 0.05$). At the 14th day, serum levels of BGP in group C were significantly higher ($p < 0.05$). After 30 days, compared with group A, the serum levels of BGP in group B and C seems to be lower. Compared with group A, the serum levels of ALP in group B and C were significantly higher, whereas the serum levels of ALP in group C seems to be higher than B ($p < 0.05$).

Conclusions: The present results suggest that injection of delayed release microsphere of exenatide on ZDF rats can release exenatide at a steady rate and the blood glucose can be controlled at a constant level. Implant survival rates could be enhanced in DM subjects when blood plasma glucose level is under control; the serum levels of BGP in this study seems to have no relationship with local osseointegration, whereas the serum levels of ALP might offer insights into the activity of osseointegration around the implant surface.

KEY WORDS: alkaline phosphatase, dental implant, diabetes mellitus, glycemic control, hyperglycemia, osseointegration, osteocalcin

INTRODUCTION

As osseointegration of dental implant was initially introduced in dentistry by Branemark in 1960s, dental implants have been successfully used to reconstruct missing teeth and showed a higher success rate. However, many systemic diseases such as osteoporosis, diabetes, and autoimmune diseases may inhibit the bone-implant integration in clinical practice, and even worse may induce to a failure of dental implant treatment, and so the further use of this technique was impacted.[1] Studies have shown that implant success rates in diabetic patients are lower than that in healthy patients; implants in patients with diabetes mellitus are likely to have a higher failure rate and poorer initial osseointegration.[2,3]

Diabetes mellitus (DM) is characterized by hyperglycemia due to insufficient insulin action or impaired insulin secretion. It is a metabolic disorder characterized

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by hyperglycemia associated with a wide range of disorders, such as retinopathy, nephropathy, cardiovascular disease, osteoporosis, impaired wound and bone healing, and increased susceptibility to periodontal disease.[4,5] Moreover, diabetes-associated alterations in microvascularization can lead to a diminished immune response and a reduction in bone remodeling.[6] All of the above systemic changes can impair the insertion and osseointegration of dental implants.

Although the majority of studies suggested that DM could negatively interfere with the process of dental implant osseointegration, even result in implant loosening and failure due to incomplete and delayed bone formation around the implant.[7–10] Some studies demonstrated that implant survival rates could be enhanced in DM patients when blood plasma glucose level is under control.[11,12] It seems certain that induced diabetes affects bone metabolism generally; specific effects around implanted biomaterials require additional study.

The key thing for the initial stability of dental implant is early osteoblasts attachment on implant surface. Osteocalcin (bone Gla protein [BGP]) is vitamin K-dependent calcium-binding protein synthesized by osteoblast and found primarily in bones.[13] The protein contains three residues of the amino acid gamma-carboxyglutamic acid (Gla) which, in the presence of calcium, promotes binding to hydroxyapitite and subsequent accumulation in bone matrix.[14] As BGP synthesized from bone tissue, half of it deposited in the bone matrix and half in blood circulation, so serum osteocalcin measurements can provide a noninvasive specific marker of bone metabolism.[15] Alkaline phosphatase (ALP) is another bone formation marker. Studies have proved that persistent hyperglycemia can inhibit osteoblast differentiation,[16,17] reduce alkaline phosphatase activity, and delay bone deposition and mineralization.[18]

In this study, we aimed to investigate the impact of DM and different glycemic control times on early osseointegration of dental implants and to explore possible mechanism by expression and significance of serum BGP and ALP levels in Zucker diabetic fatty (ZDF) rats after implantation.

METHODS

Animal Preparation

Animals were maintained in an SPF facility (Shandong Lvye Pharmaceutical Co., Ltd, Yantai, Shandong, China); protocols were approved by the Institutional Animal Use Review Board. For this study, 33 male ZDF rats 3 months old and weighing 450 g at the beginning of the experiments were utilized. Rats were housed with a 12-hour light/dark cycle and allowed a standard pellet diet and tap water ad libitum throughout the observation period. The animals were divided into three groups, each group with 11 rats and each rat with two dental implants (33 rats and 66 implants in total): group A, diabetic rats with 22 dental implants (controls); group B, diabetic rats treated with exenatide and 22 implants placed simultaneously; and group C, diabetic rats treated with exenatide until serum glucose at a constant level and then all the 22 implants be placed. Animals in group C received a subcutaneous injection of delayed release microsphere of exenatide (0.74 mL/100 g, 0.1 mL/100 g of weight, Shandong Lvye Pharmaceutical Co., Ltd); this kind of microsphere releases exenatide at a steady rate, so we just need to use it every 7 days until the end of this project. Fifty days later, as soon as the blood glucose was controlled at a constant level (≤16 mmol/L), dental implants were inserted. Animals in group B also received a subcutaneous injection of delayed release microsphere of exenatide (0.74 mL/100 g, 0.1 mL/100 g of weight, Shandong Lvye Pharmaceutical Co., Ltd); at the same time, dental implant were inserted simultaneously. Control animals received an injection of saline only. Blood glucose was detected during the whole period by blood samples obtained from the animal's tip tail.

Implantation

Rats were anaesthetized with a peritoneal injection of 4% sodium pentobarbital (0.3 mL/100 g body weight). A full-thickness incision was performed on the anteromedial portion of the femur, and the implant site was prepared using a 2.3-mm-diameter drill under constant irrigation with sterilized physiological saline solution. We then inserted the implant (SLA coated, screw, 2.5 × 2 mm, Dentium®, Seoul, Korea) and confirmed its stability by passive mechanical retention. The wound was closed with conventional sutures. After the surgical procedure, the rats received a single dose of 0.06 mL/kg of penicillin via intramuscular injection for 3 days.

Blood Glucose Level

General conditions of animals were monitored; all of the rats in three groups were weighed every 7 days during the course of the experiment. Blood glucose was detected during the whole period by blood samples obtained from the animal's tip tail.

Expression of Serum BGP and ALP Levels

Rats were sacrificed at 1, 2, 4, and 8 weeks after implant surgery in batches. Blood samples taken at the time of sacrifice were analyzed for serum osteocalcin and alkaline phosphatase. Osteocalcin was measured using an enzyme-linked immunosorbent assay technique specific for rat osteocalcin (Sigma-Aldrich Co. LLC., Shanghai, China). Alkaline phosphatase was measured using p-Nitro phenyl phosphate colorimetric determination (Sigma-Aldrich Co. LLC.). All tests were performed per manufacturers' instructions.

Statistical Analysis

The software program SPSS (version 17.0 for Windows, SPSS Co., Wuhan, China) was used to analyze all data. Differences among groups and days were evaluated; *t*-tests for differences between the three groups were performed. A p value less than 0.05 would have been considered statistically significant.

RESULTS

General Conditions of Animals

All of the animals were in good condition and did not present any disturbances on soft tissue healing or tibiae fractures. In group A, two of 22 implants was lost because of peri-implantitis; the success rate is 90.9%. In groups B and C, the success rate was 100% separately; there was significant difference. Table 1 shows the fluctuation of animals' weight of the three groups over time during the course of the experiment; DM rats treated with exenatide gained weight, whereas the control animals continued to lose weight; animals in group A weighed significantly less than groups B and C ($p < 0.05$). Blood glucose levels were shown in Figure 1; glucose of animals with delayed release microsphere of exenatide was controlled at a constant level in groups B and C, and there is significant difference between group A and other two groups ($p < 0.05$).

TABLE 1 Animals' Weight of the Three Groups Over Time

Group	*n*	Weight (g)	F	*p*
A	11	362.9 ± 21.4	900.89	0.001
B	11	497.1 ± 17.5		
C	11	501.6 ± 18.7		

Note. The baseline of all animals' weight is 400 ± 12.3 g. With time goes, weight of animals in group A decreased, whereas weight of animals in groups B and C raised. Compared with groups A and B, groups A and C, group A weighed significantly less, whereas weight of groups B and C without significantly difference.

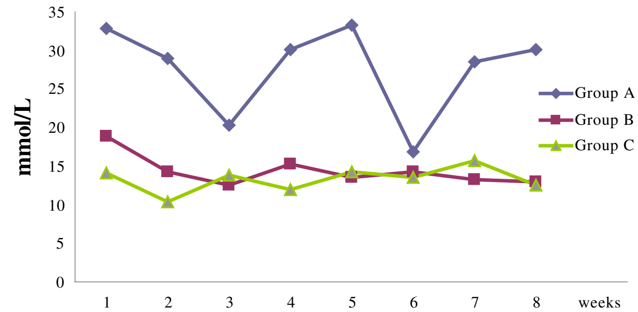

Figure 1 Blood glucose levels. Group A, control group with DM rats. Groups B and C, animals with delayed release microsphere of exenatide, there is significant difference between group A and other two groups ($p < 0.05$).

Expression of Serum BGP and ALP Levels

At the 7th day, serum levels of BGP in groups B and C were lower than that in group A; the difference was not statistical ($p > 0.05$). At the 14th day, serum levels of BGP in group C were significantly higher ($p < 0.05$). After 30 days, compared with group A, the serum levels of BGP in groups B and C seems to be lower; the difference was not statistical ($p > 0.05$) (Figure 2).

The serum levels of ALP: compared with group A, groups B and C significantly increased, and the difference has statistical meaning; the serum levels of ALP in group C was significantly higher than that in group B ($p < 0.05$) (Figure 3). With time goes, the serum levels of

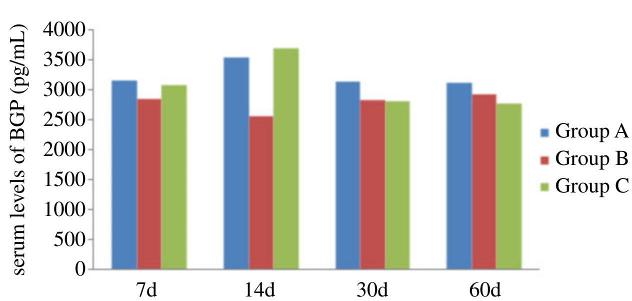

Figure 2 Serum levels of BGP at 7 days, 14 days, 30 days, and 60 days after implantation. At the 7th day, serum levels of BGP in groups B and C were lower than that in group A ($p > 0.05$). At the 14th day, serum levels of BGP in group C were significantly higher ($p < 0.05$). After 30 days, compared with group A, the serum levels of BGP in groups B and C seems to be lower ($p > 0.05$).

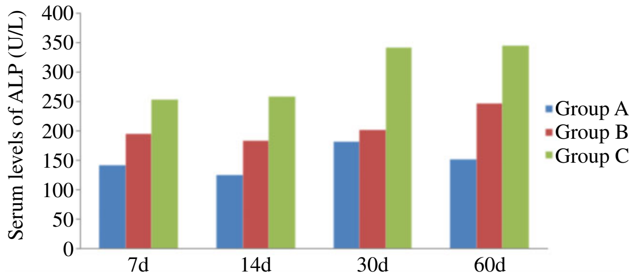

Figure 3 Serum levels of ALP at 7 days, 14 days, 30 days, and 60 days after implantation. Compared with group A, groups B and C significantly increased, and the difference has statistical meaning; the serum levels of ALP in group C was significantly higher than that in group B.

ALP in groups B and C tend to increase; however, at 60 days in group A, the level has a downward trend; the difference was not statistical ($p > 0.05$) (Figure 4).

DISCUSSION

The influence of DM on dental implants has been widely studied in recent years. Accordingly, DM remains a relative contraindication for implant therapy, as it can cause delayed healing, unstable fibron integration, and infections. Treatment can fail because of premature loss of the implant or defects in osseointegration, leading to eventual implant failure. Hasegawa[19] reported that bone volume around implants was consistently (from weeks 4–8 postimplantation) smaller for the diabetes group than for the control group in the cortical area. Bone-implant contact percentage was considerably lower for the diabetes group in both the cortical and marrow areas, with the week 4 bone-implant contact in the cortical area being 12% for the diabetes group and 61% for the control group. A twofold difference remained at week 8. Bone morphogenesis in the diabetic rats was characterized by fragmented bone tissues and extensive soft tissue intervention. So blood plasma glucose level should be the first consideration to DM patients who need dental implants treatment. In various retrospective studies,[20–22] the observed implant success rates at 1 year after implantation in diabetic patients with controlled blood glucose level ranged from 88.8% to 97.3%, the implant success rates at 1 year after restoration ranged from 85.6% to 94.6%,[23,24] and it is acceptable in the clinic. In this study, the success rate of group A was 90.9%, and 100% in group B and C. ZDF rats with controlled blood glucose level tend to have higher success rates.

Previous studies have reported controversial results about insulin therapy, and some authors reported that insulin therapy on dental implants can regulate and reduce the effects of diabetes on bone healing and result in more bone formation.[25–27] However, the traditional method of administration cannot control release rate, so it need to be administrated frequently; in recent years, biodegradable microspheres have received more and more attentions for therapeutic application such as controlled release and drug targeting[28]; the in vitro release of microspheres was usually achieved using the centrifuge instead of the dialysis method.[29,30] So in this study, we used a delayed release microsphere of exenatide; it has been proven preclinical that glucose can be controlled at a constant level by once a week of injection of this kind of microspheres on db/db and ZDF rats for 4 weeks (Shandong Lvye Pharmaceutical Co., Ltd). In our study, we used ZDF rats. The ZDF rat is an accurate model for type 2 diabetes based on impaired glucose tolerance caused by the inherited obesity gene mutation that leads to insulin resistance. Animals in groups B and C received a subcutaneous injection of delayed release microsphere of exenatide every 7 days until the end of this project; the results showed that blood glucose in treated groups was well controlled, and blood glucose fluctuation was small, whereas the controlled group A had no change.

Glucose blood levels and weight changes in our study confirmed onset of diabetic symptoms.[3] The diabetic animals in group A had significantly higher blood glucose levels and increased weight loss. The diabetic animals controlled by exenatide in groups B and C were not significantly different than controls for these parameters.

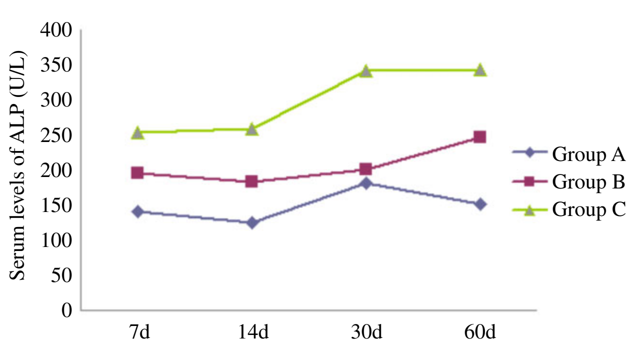

Figure 4 Serum levels of ALP. With time goes, the serum levels of ALP in groups B and C tend to increase. However, at 60 days in group A, the level has a downward trend; the difference was not statistical ($p > 0.05$).

Osteocalcin is a calcium-binding protein of bone, involved in bone mineralization and calcium homeostasis.[31] It serves as a marker for bone turnover and is typically depressed in diabetic subjects because of a general decrease in bone cell activity.[32] In this study, at the 7th day, serum levels of BGP in groups B and C were lower than that in group A; the difference was not statistical ($p > 0.05$). At the 14th day, serum levels of BGP in group C were significantly higher ($p < 0.05$). After 30 days, compared with group A, the serum levels of BGP in groups B and C seems to be lower; the difference was not statistical ($p > 0.05$); the correlation between serum osteocalcin levels and localized bone healing was not reflected. This might contribute to that the systemic presents in diabetic animals have overshadowed any contribution that may be made by local bone activity around the implant. Moreover, we think another possible reason for this result maybe that the quantity of animals in each group is not enough because of the charge, so further studies with large samples should be needed.

Alkaline phosphatase also serves as an indicator of bone formation as it is produced by cells that differentiate into osteoblasts;[32] it is commonly used as a measure of cytokine impact on osteoblasts. One article reported a systemic increase in the levels of serum alkaline phosphatase in diabetic rats relative to controls[33] and suggested that the increase in serum alkaline phosphatase seen in diabetic rats may result from an increased production of intestinal alkaline phosphatase. However, in our study, serum ALP levels in ZDF rats treated with exenatide were significantly higher than in controls; therefore, the increased bone production around the implants in groups B and C is reflected in serum ALP levels compared with group A. With time goes, the serum ALP levels also tend to be increased along with osseointegration of dental implants in all the three groups, so the correlation between serum levels of ALP and localized bone healing was realized. According to the results, serum ALP levels in group C are significantly higher than in group B which suggest that osseointegration in well-controlled blood glucose in ZDF rats was better than diabetic rats treated with exenatide and implants placed simultaneously. The increased levels of alkaline phosphatase in treated groups suggest that control of blood glucose in DM subjects can efficiently improve bone activity.

CONCLUSIONS

The present results suggest that injection of delayed release microsphere of exenatide on ZDF rats can release exenatide at a steady rate and the blood glucose can be controlled at a constant level. Implant survival rates could be enhanced in DM subjects when blood plasma glucose level is under control; the serum levels of BGP in this study seems to have no relationship with local osseointegration, whereas the serum levels of ALP might offer insights into the activity of osseointegration around the implant surface.

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