Calcium, Phosphorus and Oxygen Around Implant at Early Osseointegration in Hyperlipidemic Rats

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Abstract: The study was to observe dental implant osseointegration at early stage and analysis the influence of the ratio of calcium and phosphorus variation on osseointegration in hyperlipidemic rats. Wistar rats were randomly divided into control group with normal diet and experimental group with high fat diet. 8 weeks later, serum lipid levels was detected and titanium implants were placed into bilateral femurs. At day 1, 3, 5 after implant, rats were sacrificed and 5 mm bone with implants was obtained. hard tissue slices and methylene blue-acid fuchsin staining were performed to observe the interface of bone-implant. And energy disperse X-ray spectrometer (EDS) was used to measure the percentage of oxygen and the ratio of calcium and phosphorus. All rats in experimental group were successfully induced into hyperlipidemic status. Histological results revealed less osteoblasts and trabecular bone, but more osteoclasts and trabecular space in experimental group than those in control group. Higher content of oxygen and lower ration of calcium and phosphorus were also observed in experimental group than those in control group (P<0.05). Hyperlipidemia could hinder implant osseointegration at early stage after implantation. This inhibition might be closely related to higher content of oxygen and lower ratio of calcium and phosphorus.

Key Words: Bone healing, Hyperlipidemia, Implant osseointegration, Oxygen, Ratio of calcium and phosphorus

Introduction

Hyperlipidemia is characterized with elevated lipids in bloodstream, which is caused by atherosclerosis or high-fat (HF) diet. Hyperlipidemia is closely related to osteoporosis, which usually involves alveolar bone. And mice with HF diet demonstrate significant increased implant failure, decreased bone formation and compromising strength of implant-bone interface. In recent years, the morbidity of hyperlipidemia is increasing. In America, 12.1% of adults had high total cholesterol (TC) and 18.5% with low high-density lipoprotein (LDL) cholesterol during 2011-2014.

With the survive rate of dental implant is high, it becomes common prosthetics in the world. The successful osseointegration is determined the long-term survive rate. The studies about the influence of hyperlipidemia on implant osseointegration is still at an initial stage. Some studies have indicated that hyperlipidemia inhibits osteoblastogenesis and bone metabolism through hindering alkaline phosphatase activity and mineralization, which were markers of osteoblast differentiation. Hyperlipidemia also impaired bone regeneration and mechanical strength.

Oxygen (O) is necessary in cell metabolism and bone repair by regulating proliferation and differentiation of bone marrow stem cells (BMSCs), chondrocytes and osteoblasts. O participates oxidation-reduction reaction to produce reactive oxygen species (ROS). High lipid level could increase oxidative stress by overproduction of ROS that bone healing process between the implant-bone interface was suppressed. Except O, calcium (Ca) and phosphorus (P) are also important components in bone mineralization and bone formation. Ca has been proved to be crucial in implant osseointegration, involving platelet adhesion, orientation and conformation of proteins on the interface. Low concentration of P is released from bone, which simultaneously lead to the release of Ca because of an unfavorable ratio of Ca to P. Interaction between Ca and P could affect bone metabolism and development. The Ca/P was positively related to bone density, strength and quality. Maintaining an adequate Ca and P with appropriate ratio is of crucial importance for implant-bone interface stability and good osseointegration. But the effect of hyperlipidemia on O, Ca and P is still unknown.

The poor implant osseointegration at implant-bone interface at the early stage after implantation resulted in the majority of dental implants failure. In this study, we observed implant osseointegration at implant-bone interface of hyperlipidemic rats from histological slices. Meanwhile, O, Ca and P in tissue were also assayed using EDS.
Materials and Methods

The study was approved by the ethics committee of Shandong University Dental Hospital, and informed consents were obtained (protocol number GD201615).

Animals

36 adult male Wistar rats were obtained from Shandong University Experimental Animal Center (Ji’nan, China). All experimental protocols were guided and approved by Institutional Animal Care and Use Committee of School of Stomatology of Shandong University (Ji’nan, China). The rats were randomly divided into control group fed with normal diet and experimental group with HF diet during whole experiment. HF diet was purchased from Beijing Ke’aoxieli Co., Ltd (Beijing, China).

8 weeks later, endocanthion vein blood specimens were collected to monitor serum TC, Triglyceride (TG), high-density lipoprotein (HDL) and LDL.

Implantation and samples preparation

All rats were anesthetized with chloral hydrate (0.3 ml/100 g). After regular skin preparation and disinfection, a 1 cm vertical incision was made near the epiphyseal of femur and blunt dissection of femoral rectus and femoral muscles to fully expose bone. The implant hole was prepared by 1.2 mm pilot drill, which rotated at 800 revolutions per minute and cooled with saline. Titanium (Ti) implants (Shuangyang®...
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At day 1, 3, 5 after implantation, the rats from each group were sacrificed by overdosed chloral hydrate. 5 mm bones around implants were dissected and fixed in 4% paraformaldehyde, dehydrated in ascending series of ethanol and embedded with resin.

Hard tissue slices and staining

The samples were cut and grinded into 30 μm thick sections (E400CS, EXAKT Vertriebs GmbH, Germany), then methylene blue staining was performed. Inverted microscopy was used to observe and photograph the interface of implant-bone. Image-Pro Plus software was used to analyze the area of trabecular bone and the number of osteoblasts in the original photograph at day 1, 5 after implantation and made a histogram.

Micro-composition analysis

Other samples of each group were used to measure concentrations of O, Ca and P at six microzones, and calculate percentage and ratio with energy disperse X-ray spectrometer (EDS). Six microzones were

self-tapping screw, 1.5 mm wide and 2.5 mm long, Zhangjiagang city, China) were placed into bilateral femurs distal to epiphyseal. Mucosa and muscles were sutured with 4-0 thread and skin with 5-0. 200000 u Cefazolin sodium was administrated in 3 days after surgery.

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Other samples of each group were used to measure concentrations of O, Ca and P at six microzones, and calculate percentage and ratio with energy disperse X-ray spectrometer (EDS). Six microzones were
marked “1~6” at 10 μm intervals and perpendicular to the medium 1/3 of implants (Fig. 1). “3” and “4” were around the edge of implant.

Statistical analysis
Data were expressed as mean ± standard deviation (SD) and analyzed by SPSS software 13.0. Comparisons were analyzed with One-way ANOVA and t-test. P<0.05 was considered statistically significant difference.

Results
Establishment of hyperlipidemic rats
TC level in experimental group was about twofold than that in control group (P<0.05), and also TG, HDL and LDL levels were significant higher than those of control group (P<0.05) (Table 1, Fig. 2). All rats in experimental group were successfully induced to hyperlipidemic rats.

Analysis of hard tissue slices
At day 1, 3 and 5 after implantation, there were crescent-shaped trabecular bone, bone remodeling caused by osteoclast phagocytosis, and gradually increased osteoblasts, osteoclasts and collagen fibers. There is a large amount of osteoclasts distribution, with three or more nuclei in control group (Fig. 3A). In experimental group at day 1, 3, 5 (Fig. 4B, D and F), trabecular bone arranged disorder, and collagen fibers were less around the implant.

Through the quantitative analysis of the slices, there were more trabecular bone (Fig. 4B) and osteoblasts (Fig. 4A), less osteoclasts (Fig. 4A) and collagen fibers (Fig. 4B) in control group than those in experimental group at day 1, 5 after implantation. It can be seen that there is a statistically significant difference between the control group and the experimental group (*P<0.05).

Micro-composition analysis
In both groups, the percentage of O increased a little with time at microzone 5 and 6, but decreased at microzone 4 (P<0.05) (Table 2, Fig. 5). Percentage of O in experimental group was significantly higher than that in control group at microzone 4, 5, and 6 (P<0.05).

Ca/P increased slightly from day 1 to day 5 in both groups at microzone 4, but decreased at microzone 5 and 6 (P<0.05) (Table 3, Fig. 6). Ca/P in experimental group was significantly lower than that in control group at microzone 4, 5, and 6 (P<0.05).

Discussion
In this study, we tested the influences of hyperlipidemia on dental implants osteointegration historically in rats at early stages (1st, 3rd, 5th day post-surgery). In our results, osseointegration at early stage was hindered in hyperlipidemic rats and this inhibition might be closely related to higher content of oxygen and lower ratio of calcium and phosphorus than normal rats.

Osteoblasts, which play a key role in osseointegration process, secrete bone matrix with type I collagen as its main component. The deposition of calcium and phosphorus on collagen fibers forms hydroxyapatite to mineralize bone matrix. Osteoblasts are surrounded by newly forming tissue to become bone cells, which in turn produce new bone tissue. Ca and P ions around the implant produce an initiating stimulation for osteoblasts resulting in an increasing of the amount of synthetic collagen and ultimately promotes the formation of new bone. In this study, we found that although the number of osteoblasts and trabecular bone around the two groups increased with time, the composition of bone cells and trabecular bone was less in hyperlipidemia group. There were more osteoclasts and trabecular space than the control group.

HF diet could induce the changes of bone morphological and mechanical properties, such as bone density and content of bone mineral, and affect implant osseointegration. The osseointegration is similar to bone fracture healing. During fracture healing, osteoblasts deposit collagenous matrix. The first day after implantation, mesenchymal cells, pre-osteoblasts and osteoblasts adhered to implant surface and began to deposit collagen matrix directly on the early formed cement line/lamina limitans layer described on the implant surface. Hyperlipidemia disrupted collagen processing as well as orientation, causing loss of local alignment, which might reduce mechanical integrity and quality of bone.
and impair bone healing. Epidemiological data showed that HF diet could increase lipoprotein levels and their oxidative products, enhancing oxidative stress. Intracellular oxidative stress was the initial factor for changing of bone metabolism. Oxidative stress not only led to inhibition of osteogenic signaling pathway, but also decreased formation of mature osteoblasts and expression of molecular markers of bone remodeling. Serum lipid levels had an negative correlation with the bone mineral content, bone mineral density and mass. It was confirmed that oxidative stress induced by ROS at implant-bone interface greatly reduced cells growth onto the implant surface and biological functions of osteoblasts, which would hinder bone repair at early and later stage, even lead to implant failure. Higher content of O in experimental group induced overproduction of ROS, which decreased osteoblasts and new bone formation in hyperlipidemia.

Ca had principal roles at almost every stage of bone repair process: from the formation of the provisional matrix to mineralization and bone remodeling. Studies in vitro showed that increased concentrations of Ca and P ions in the medium contributed to proliferation and differentiation of pre-osteoblasts and MSCs to osteoblasts, leading to new bone formation. Ti implant surfaces modified with Ca ions stimulated osteoblasts attachment, proliferation and differentiation, and improved implant osseointegration. In addition, Ca and P ions-co-immobilization onto Ti implant could stimulate the response of pre-osteoblasts, with greater alkaline phosphatase activity and bone-like nodule formation.

A normal range of Ca/P will lead to optimal bone mineralization and bone formation. The Ca/P of native bone hydroxyapatite (HA) is 1.67 in human, so most bone related bioactive materials aims at this critical range. A normal range of Ca/P will lead to optimal bone mineralization and bone formation. A normal range of Ca/P will lead to optimal bone mineralization and bone formation.

Ca/P ratio of 1.57 to 1.71, which are closer to the Ca/P of native bone HA, could be beneficial to bone mineralization and formation. Furthermore at early calcification of nodules, 1.35 was good for the formation of the provisional matrix to mineralization and bone remodeling.

In conclusion, hyperlipidemia hindered implant osseointegration in rats at early stage after implantation. This inhibition might be modulated by overproduction of oxidative stress and low Ca/P, which further affected osteoblast differentiation, bone mineralization and formation.

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Conflict of Interest

There are no conflicts of interest in this study.

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