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Research Paper
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Insulin promotes bone formation in augmented maxillary sinus in diabetic rabbits

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Abstract. The role of insulin during the formation of bone in the augmented space of the maxillary sinus in patients with diabetes is unclear. The authors compared the differences in bone formation after maxillary sinus floor elevation in diabetic and healthy animals and evaluated the effects of insulin on osteogenesis and the differentiation and activities of the osteoblasts. 10 male Japanese white rabbits were divided into two groups after diabetic induction by a single injection of monohydrated alloxan and having maintained steady blood glucose levels. The groups included the diabetes mellitus group (DM; $n = 5$) and the DM + insulin group ($n = 5$); another five healthy rabbits comprised the control group. Maxillary sinus floor elevation was performed by grafting hydroxyapatite particles. Compared with the control group, the newly formed bone area, number of blood vessels and osteoblasts, collagen I content and serum osteocalcin levels were significantly decreased in DM rabbits ($P < 0.01$). Insulin treatment reversed the decrease in bone formation, blood vessels, osteoblasts, collagen I and serum osteocalcin ($P < 0.01$). Insulin treatment also promoted osteogenesis in the augmented space of the diabetic rabbits, which might have resulted from promotion of osteoblast differentiation and upregulation of neovascularization.

Key words: diabetes mellitus; bone formation; maxillary sinus floor elevation; osteocalcin; osteoblast..

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The maxillary sinus graft procedure, also referred to as maxillary sinus floor elevation, is a well-established technique for increasing bone volume in the posterior edentulous maxilla before dental implant insertion[22,23,26,30,31]. This technique has been performed by increasing numbers of implant surgeons because of its effectiveness and relatively low risk, but it is now known that uncontrolled diabetes is a contraindication for dental implants[18]. Although the relationship between diabetes and dental implants has been reviewed[11], relatively little is known about the influence of diabetes on the success rate of maxillary sinus floor elevation. Studies using animal models have shown that diabetes leads to reduced bone formation, which results in osteopenia and delayed fracture healing[15]. Previous studies exploring the influence of diabetes mellitus (DM) on bone formation have adopted models of experimental fracture of the tibia or femur[3,7,14] and found that DM delays fracture healing and that insulin can reverse this effect[3]. Bone formation in the limb bones is mainly through endochondral ossification, whilst bone formation in

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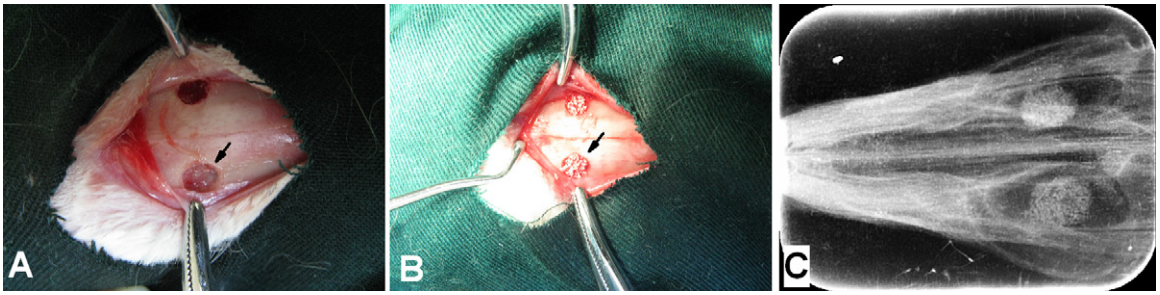

Fig. 1. Surgical procedure for maxillary sinus floor elevation. (A) Two bone windows with diameter about 4 mm were made on the nasal bones. The translucent sinus membranes were seen through the windows. (B) Mixture of hydroxyapatite and blood is grafted into the maxillary sinus. (C) X-ray of bone formation in the maxillary sinus after the rabbits were killed.

the jawbone is mainly through intramembranous ossification. The changes in bone formation in the augmented space of the maxillary sinus in patients with diabetes remain unknown.

Intramembranous ossification is characterised by direct osteoblastic differentiation without cartilage formation. In the present study, the authors investigated the number of active osteoblasts and their functional activities, including serum osteocalcin levels and collagen I content.

To produce a type I diabetic model, the authors intravenously injected monohydrated alloxan to impair the rabbits' pancreatic β cells. In this model, maxillary sinus grafting was performed with and without insulin treatments. The aim of the present study was to compare the differences in bone formation after maxillary sinus floor elevation in diabetic and healthy animals and to evaluate the effects of insulin on osteogenesis and the differentiation and activities of the osteoblasts.

Materials and methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, China. 6-month-old male Japanese white rabbits (body weight 2.5–3.0 kg) were obtained from the Animal Centre of the Affiliated Second Hospital, Harbin Medical University, China, and were bred in the animal facilities at Harbin Medical University.

Monohydrated alloxan, pentobarbital sodium, and insulin were obtained from Sigma (St. Louis, MO, USA). Mouse anti-rabbit monoclonal antibodies to osteocalcin were obtained from Abcam (Hong Kong, China). Hydroxyapatite ceramic particles were obtained from Beijing YHJ Science and Trade Co. (Beijing, China). Collagen membranes (Bio-Gide) were obtained from Geistlich (Wolhusen, Switzerland).

Induction of the rabbit diabetic model and experimental design

All the animals ($n = 21$) were kept for 2 weeks to adapt to their environment. Blood samples were collected from the auricular veins of the animals for the evaluation of basal serum glucose levels using the glucose-oxidase enzymatic method. Five rabbits comprised the control group and the other 16 rabbits were subjected to the diabetic model.

In the experimental groups, diabetes was induced by a single intravenous injection of 150 mg/kg of body weight monohydrated alloxan (Sigma, St. Louis, MO, USA) dissolved in sterile 0.9% saline. Within 12 h of alloxan administration, a 5% glucose solution was offered to the animals to prevent hypoglycemia.[10,25] At two time points, 72 h and 3 weeks after alloxan administration, the serum glucose levels were determined. Animals with serum glucose levels at or above 16.67 mmol/L at both time points were considered diabetic, and those with serum glucose level below 16.67 mmol/L were excluded from the study. Diabetes was successfully induced in 10 rabbits (from a total of 16) and they were randomly divided into two groups: the DM group ($n = 5$) and the DM + insulin group ($n = 5$).

Surgical procedures

After confirmation of the diabetic model 72 h and 3 weeks after alloxan administration, the rabbits underwent the surgical procedure. The hair was shaved and the surgical area was sterilized with iodophors and alcohol. The rabbits were anaesthetized by intravenous injection of pentobarbital sodium (1.5 mg/kg) dissolved in sterile 0.9% saline, and 0.5 ml of 1% lidocaine with epinephrine (1:100,000) was injected subcutaneously at the midline of the nasal dorsum for local anaesthesia. A 3 cm vertical midline incision was made as described by Asai et al.[1], and the skin and periosteum were elevated sufficiently to expose the nasal bone and nasoincisal suture line. Two circular nasal bone windows (diameter 4 mm) were made as shown in Fig. 1A. The maxillary sinus membrane was kept intact during the procedure. The membrane was gently pushed inward and elevated from the floor, lateral wall, and medial wall of the maxillary sinus, and a compartment for graft materials was obtained[1,30]. The hydroxyapatite ceramic particles (diameter 400–1000 μm; Beijing YHJ Science and Trade Co., Beijing, China) were mixed with the blood collected from the auricular vein of each rabbit, packed into the compartment, and slightly compressed (Fig. 1B). The bone windows were covered with a collagen membrane (Bio-Gide; Geistlich, Wolhusen, Switzerland) to prevent fibrous connective tissue ingrowth. The skin was then tightly sutured. A total of 200,000 U/day of penicillin was injected intramuscularly for 1 week after the operation. Figure 1C shows the X-ray image of bone formation in the maxillary sinus after the rabbits were killed. The serum glucose levels were determined every week after the operation. Before the animals were killed, a blood sample was collected from the left ventricle for the last evaluation of serum glucose levels.

Insulin treatment

The DM + insulin group received twice-daily subcutaneous injections of swine insulin (10 U/day; Sigma, St. Louis, MO, USA). The rabbits from the control group and the DM group were injected with a sterile saline solution[17].

Tissue preparation

The animals were killed at the end of the eighth week after surgery. The rabbits were anaesthetized intravenously with pentobarbital sodium (Sigma, St. Louis, MO, USA). After 20 ml of blood was