VETERINARSKI ARHIV 80 (1), 113-120, 2010

Influence of hydroxyapatite on fracture healing in diabetic rats: biomechanical and radiographic studies

Abdolhamid Meimandi Parizi[1]*, Golamali Jelodar[1], Hamidreza Moslemi[2], Azizola K. Tafti[1], and Mohammad J. Emami[3]

[1]*School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

[2]*Islamic Azad University, Garmsar branch, Garmsar, Iran*

[3]*Department of Orthopedic Surgery, Shiraz University of Medical Sciences, Shiraz, Iran*

MEIMANDI PARIZI, A., G. JELODAR, H. MOSLEMI, A. K. TAFTI, M. J. EMAMI: Influence of hydroxyapatite on fracture healing in diabetic rats: biomechanical and radiographic studies. Vet. arhiv 80, 113-120, 2010.

ABSTRACT

Patients with diabetes mellitus incur a high incidence of fractures. This suggests that the structural integrity of the skeletal system may be compromised. This study was conducted to evaluate the effects of hydroxyapatite (HA) on fracture healing in diabetic rats by biomechanical and radiographic methods. Twenty-four adult male rats were randomly divided into four identical groups. Diabetes was chemically induced in 2 groups by alloxan. After 2 weeks, all the rats were anesthetized and a transverse osteotomy of the right radius was performed under aseptic conditions. In one diabetic and one non-diabetic group, the osteotomy gap was filled with HA while the other 2 groups did not receive HA. Radiographs were taken at 0, 15 and 35 days post operation. All the rats were euthanized 5 weeks post operation, and the radial bones were harvested and prepared for the three-point bending test. Comparison between the HA treated groups (diabetic and non diabetic), demonstrated that diabetes significantly reduced the capacity of bone to loaded bending forces. The non-treated diabetic group showed fewer radiographic signs of union and remodeling of fractures in comparison with the other three groups. In conclusion the diabetic rats that did not receive HA exhibited inferior biomechanical properties and radiographic signs in comparison with the HA treated groups and the negative control group.

Key words: hydroxyapatite, fracture healing, diabetic rat, biomechanics, radiography

Introduction

Fracture healing is a process of restoring the structural and biological properties of injured bone. Osteopenia is one of the most common complications in diabetic patients. Diabetic osteopenia has been reported in many clinical human and experimental animal

*Corresponding author:

Abdolhamid M. Parizi DVM, PhD, Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz 71345, Iran, Phone: +091 77117165; Fax: +0711 2286 940; E-mail: maimanp1@yahoo.com; meimandi@shirazu.ac.ir

ISSN 0372-5480
Printed in Croatia

studies. Typical findings in these patients are alterations in calcium, phosphate and bone metabolism, and reduced bone biomechanical properties (DIXIT and EKSTROM, 1987; RONALDO et al., 2003). Osteopenia occurs in association with diabetes type 1 (RONALDO et al., 2003). Evidence suggests that the development of osteopenia in diabetics could be related to increases in musculoskeletal afflictions. Different animal models that mimic the human diabetic condition have been used to elucidate diabetes-induced alterations in bone and other organs (REDDY et al., 2001). The calcium phosphate group is the largest, most important inorganic part of the hard tissues constituting bones and dentine material in vertebrate animals. Synthetic calcium phosphate or hydroxyapatite (HA) has been shown to be quite similar to the natural component of bone. It has several medical applications, such as the appropriate replacement of bony and periodontal defects (PRAMANIK et al., 2007; WOODARD et al., 2007). Several studies have shown that diabetic bones are weaker (COZEN, 1972; WEISS and REDDI, 1980; DIXIT and EKSTROM, 1987; LANCE et al., 1989; FUNK et al., 2000; HEATHER et al., 2002; HONGBING et al., 2004; REDDY et al., 2001; RONALDO et al., 2003), and there is a positive effect of HA on fracture healing (GRIFFET et al., 1999a; PRAMANIK et al., 2007; WOODARD et al., 2007). The purpose of this study was to evaluate the effects of HA on fracture healing in diabetic rats by biomechanical and radiographic examinations.

Materials and methods

Experimental design and surgical procedure. Twenty-four male Sprague Dawley rats (243 ± 17.1 g and 3 months old) were housed at a temperature of (22 ± 2 ºC) in an air-conditioned room and supplied with standard pellet food with tap water *ad libitum*. All rats received humane care according to the criteria outlined in the "Guide for the care and use of laboratory animals" prepared by the National Academy of Science and published by the National Institutes of Health.

The rats were divided randomly into four equal groups: a non-diabetic HA treated group (NDRHA), a non-diabetic non-treated with HA (NDR) group, a diabetic HA treated group (DRHA) and a diabetic group non-treated with HA (DR).

Diabetes mellitus was induced in 2 groups by the intraperitoneal injection of alloxan (Sigma Chemical Co., St. Louis, dissolved freshly in sterile saline, 0.9%) at the dose of 165 mg/kg body mass. Using an Accu-Chek advantage glucometer (BC79, Ireland), blood glucose levels were monitored during the study. The accuracy of the instrument was checked using the biochemical method (glucose oxidase). Two groups developed clinical signs of diabetes one week following the alloxan injection, with an increase in their blood glucose levels to above 200 mg/dL. In the non-diabetic groups, which were injected only with saline, the normal blood glucose level was maintained (<100 mg/dL). Two weeks after the establishment of diabetes, the rats in all groups were

anesthetized intraperitoneally with a combination of ketamine hydrochloride 10% (50 mg/kg) and xylazine hydrochloride 2% (5 mg/kg). Using a craniolateral approach, the right radius was exposed and then a transverse midshaft osteotomy was performed under aseptic conditions. In the NDRHA and DRHA groups, the osteotomy gap was filled with hydroxyapatite (OsSatura BCP, Isotis, The Netherlands) while the DR and NDR groups did not receive HA. Flunixin (Razak Co. Iran) as an analgesic was administrated (2.5 mg/kg intramuscularly) to the all rats postoperatively. Following recovery, the animals were returned to individual cages for the remainder of the experiment. The rats were weighed at the start and end of the study.

Radiographic evaluation. Radiographs were taken in lateral and cranio-caudal views at 0, 15 and 35 days post surgery. Exposure factors of 45 KV, 20 mA, 16 mAs and 70 cm FFD were used. The radiographic examination included assessment of the callus formation and the presence or absence of fracture gap. The radiographs were interpreted blindly by two specialists and graded 0 to 3. The scoring system was defined as following: 0 = nil, 1 = low, 2 = medium and 3 = high (PARIZI and MAMANPOUSH, 2003).

Biomechanical tests. Five weeks post operation, the rats were euthanized with an overdose of sodium thiopental. The radii were collected and stored at -20 ºC until used in the biomechanical test. Before testing, the bones were thawed at room temperature and kept moist during the test. The three-point bending test was performed to determine the mechanical properties of the healed osteotomised radial diaphysis. The bone was placed horizontally on two rounded supporting bars located at a distance of 17 mm, and was loaded at the midpoint of the diaphysis by lowering the third bar. The bones were loaded at a rate of 1 mm/min until fracturing occurred. Tests were performed using a universal testing machine (Hounsfield Test Equipment, H10KS, UK) equipped with an interchangeable load cell (range of forces from 0 to 500 N). The load-displacement (deformation) curves were recorded and analyzed for the following variables describing mechanical properties of the bone: maximum load (the maximum load recorded during the test; the force causing bone fracture), flexural modulus (the slope of the linear, elastic part of the load-displacement curve) and flexural strength (described as bending strength, as opposed to that measured in a compression test, which reflects bone compression strength).

Statistical analysis. Statistical significance of the differences between the groups was calculated using paired *t*-test and analysis of variance. A $P<0.05$ was considered statistically significant.

Results

The diabetic rats showed a minimal body mass gain. The average weight gain of both diabetic groups was 18.5 g, whereas it was 73.5 g in the non-diabetic groups. A

significant difference was seen in body weight gain between diabetic and non-diabetic rats (Fig. 1) ($P<0.05$). A significant gain in average body weight (32%) was observed in the non-diabetic animals, whereas this figure in the diabetic animals was just under 8% during the experimental period. These findings show the significant effect of diabetes on the growth of the rats ($P<0.05$). The mean blood glucose level of the diabetic rats at the time of euthanasia was 220 mg/dL as compared to 80 mg/dL for non-diabetic rats.

The results of the three-point bending test in the four groups are shown in Table 1. The mean maximum load of the NDR, DRHA and NDRHA groups were 35%, 24% and 87% greater than the DR group, respectively. The flexural strength of the NDR, DRHA and NDRHA groups were 65%, 99% and 175% greater than the DR group, respectively. There was a significant decrease in flexural modulus of DR with NDR (89%), DRHA (137%) and NDRHA (243%) ($P<0.05$). There was a significant difference between NDR and DRHA with NDRHA and no difference between NDR and DRHA ($P<0.05$). In general, the diabetic rats non-treated with HA exhibited inferior biomechanical properties in comparison with HA treated groups.

During the period of fracture healing, the DR group showed less callus formation at the fracture site in comparison with the other three groups. At the end of study, the fracture line removal and remodeling of osteotomy site of the DR group was less than in the other groups.

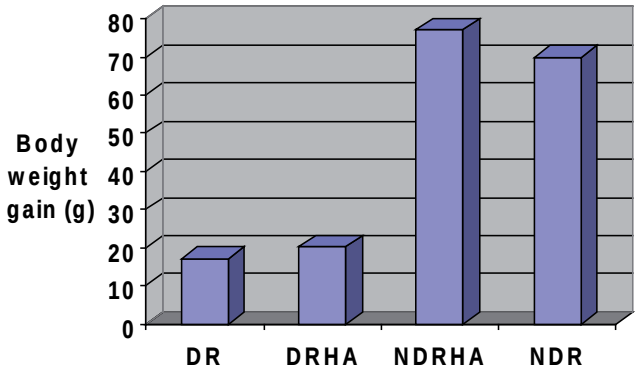

Fig. 1. Comparison of body weight gain between diabetic and non-diabetic rats during experimental period. The body weight gain rate for diabetic and non-diabetic rats was 8 and 32%, respectively. DR = diabetic rats non-treated with HA, DRHA = diabetic HA treated rats, NDRHA = non-diabetic HA treated rats and NDR = non-diabetic rats non-treated with HA. Significant difference at $P<0.05$.

Table 1. Mechanical properties of the radius in the four groups in the experiment (n = 24)

Parameters	Groups			
	DR	DRHA	NDRHA	NDR
Maximum load (N)	19.14 ± 1.50^{a}	23.81 ± 2.86^{b}	35.97 ± 8.00^{c}	25.87 ± 2.20^{b}
Flexural strength (MPa)	26.79 ± 7.63^{a}	53.42 ± 16.37^{b}	73.7 ± 22.15^{c}	44.24 ± 21.8^{b}
Flexural modulus (MPa)	494.4 ± 228.61^{a}	1174.2 ± 689.76^{b}	1694.4 ± 1153.34^{a}	934.8 ± 99.40^{b}

DR = diabetic rats non-treated with HA, DRHA = diabetic HA treated rats, NDRHA = non-diabetic HA treated rats and NDR = Non-diabetic non-treated with HA. N = Newton, MPa = Mega Pascal. Values with different superscripts in each row are those that differ significantly ($P<0.05$).

Discussion

As in previous studies (DIXIT and EKSTROM, 1987; REDDY et al., 2001), the weight gain of the diabetic rats decreased significantly compared with that of the non-diabetic animals. This is caused by the decreased availability of glucose and amino acids to cells, creating a shortage of substrates for cellular biosynthesis and affecting related cellular metabolism.

Some investigators have been studied on radial bone without any fixation method (PALEY et al., 1986; SEEHERMAN et al., 2006). Since the ulnar bone was kept intact, no stabilization was needed. The ulna supports the radius well so that fixation remains stable.

Biomechanical results demonstrated improvement of the structural strength in DRHA compared with DR and a difference between NDRHA and NDR. These results showed no difference between DRHA and NDR. Our findings revealed a significant reduction in the biomechanical parameters of radii in non-treated diabetic rats compared with diabetic HA treated rats. The radiographic findings of the current study support the biomechanical results. Bridging the gap with new bone and remodeling of healed bone was improved in HA treated rats. These results showed the delayed effect of diabetes and increased influence of HA on fracture healing, confirming the findings of some previous studies on the retardation of diabetes on bone healing in diabetic patients (COZEN, 1972; WEISS and REDDI, 1980; DIXIT and EKSTROM, 1987; LANCE et al., 1989; FUNK et al., 2000; REDDY et al., 2001; HEATHER et al., 2002; RONALDO et al., 2003; HONGBING et al., 2004) and positive effect of HA on healing of fractures (GRIFFET et al., 1999a; PRAMANIK et al., 2007; WOODARD et al., 2007).

Comparison between the HA treated groups (diabetic and non-diabetic), demonstrated that diabetes significantly reduced the capacity of bone to loaded bending forces. The difference between the diabetic groups (treated and non-treated with HA) could be related to the application of HA. Our findings support the results of GRIFFET et al. (1999b),

WOODARD et al. (2007) and PRAMANIK et al. (2007) who have studied the efficacy of HA on fracture healing and shown that the mechanical properties of synthesized HA is better than those of natural products. The altered biomechanical integrity of diabetic bones may be related to a number of factors. The physical properties of bone depend on factors such as the concentration of minerals, especially calcium and phosphate. In diabetes, there is a marked decrease in calcium absorption through the intestinal route. WEISS and REDDI (1980) reported that calcification of bone was reduced by 50% in diabetic animals. The application of calcium-phosphate composite (HA) as a graft could play a framework role at the fracture site. Although, the pathogenesis of osteopenia in diabetes is a poorly understood phenomenon, reduced bone formation and mineralization appears to be related to the poor biomechanical properties of bone in diabetes.

It is well documented that diabetes mellitus causes loss of bone mass, which may occur as a result of a decrease in the deposition of calcium (DIXIT and EKSTROM, 1987). Diabetes induces structural abnormalities that predispose bone to fractures, which may occur spontaneously or with minimal trauma in patients. Alterations in bone mass and mineral metabolism have been shown to occur in both human and experimental diabetic animals (DIXIT and EKSTROM, 1987; REDDY et al., 2001). Hydroxyapatite has been shown to be quite similar, crystallographically and chemically, to the natural materials of bone (GRIFFET et al., 1999a). Research and clinical data confirm the efficacy of this synthetic composite (WEISS and REDDI, 1980; LEMON, 1988; GRIFFET et al., 1999b). Although increased morbidity in orthopedic patients with diabetes is well documented, and in spite of the positive effect of calcium-phosphate composite in fracture healing, studies on the influence of this composite on fracture healing in diabetics are limited (EL DEEB et al., 1990; EL DEEB et al., 1991; IYAMA et al., 1997; TAKESHITA et al., 1997).

The induction of experimental diabetes in rats using chemicals which selectively destroy pancreatic Beta cells is very convenient and simple to use. The most frequently used substances to induce diabetes in rats are alloxan and streptozotocine. This type of animal model is used to induce insulin-dependent type 1 diabetes. The dosage of alloxan used in this study (single injection of 165 mg/kg body mass) maintained diabetic status throughout the study period. This is in correspondence with previous studies, where injections of alloxan above 150 mg/kg induced stable chronic diabetes in rats.

In conclusion, this study shows that the bones of diabetic rats are weaker and stiffer when compared to those of normal control rats, and the application of hydroxyapatite increased the biomechanical properties of bone in diabetic rats. Hydroxyapatite has many advantages, including biocompatibility, easy availability, shape ability, non-toxicity and non-immunogenicity. Therefore, it can be used safely in clinical cases.

References

COZEN, L. (1972): Does diabetes delay fracture healing. Clin. Orthop. 82, 134-140.

DAMIEN, E., K. HING, S. SAEED, P. A. REVELL (2003): A preliminary study on the enhancement of the osteointegration of a novel synthetic hydroxyapatite scaffold in vivo. J. Biomed. Mater. Res. A.,66, 242-246.

DIXIT, P. K., R. A. EKSTROM (1987): Retardation of bone fracture healing in experimental diabetes. Indian J. Med. Res. 85, 426-435.

EL DEEB, M., M. T. ROSZKOWSKI, I. EL HAKIM (1990): Tissue response to hydroxyapatite in induced diabetic and nondiabetic rats: histologic evaluation. J. Oral. Maxillofac. Surg. 48, 476-481.

EL DEEB, M., M. T. ROSZKOWSKI, J. SAUK, I. EL HAKIM (1991): Extracranial and mandibular augmentation with hydroxyapatite -collagen in induced diabetic and nondiabetic rats. J. Oral. Maxillofac. Surg. 49, 165-170.

FUNK, J. R., J. E. HALE, D. CARMINES, H. LEE GOOCH, S. R. HURWITS (2000): Biomechanical evaluation of early fracture healing in normal and diabetic rats. J. Orthop. Res. 18, 126-132.

GRIFFET, J., A. CHEVALLIER, E. ACCORSI, T. ELHAYEK, G. ODIN, B. PEBEYER (1999a): Osteosynthesis of diaphyseal fracture by ossatite:experimental study in rat. Biomaterials 20, 511-515.

GRIFFET, J., A. CHEVALLIER, T. ELHAYEK, G. ODIN B., PEBEYER, E. ACCORSI (1999b): Diaphyseal fracture treated by polylactide and hydroxyapatite pins. Experimental study. J. Med. Sien.: Mater. in Med. 10, 411-418.

HEATHER, A., J. R. P. BEAM, S. L. SHELDON (2002): The effect of blood glucose control upon fracture healing in the BB Wistar rat with diabetes mellitus. J. Orthop. Res. 20, 1210-1216.

HONGBING, H., L. RONGKUN, D. TESFAHUN, L. CATALDO, C. LOUIS, T. DANA (2004): Diabetes causes decreased osteoclastogenesis, reduced bone formation, and enhanced apoptosis of osteoblastic cells in bacteria stimulated bone loss. Endocrinol. 145, 447-452.

IYAMA, S., F. TAKESHITA, Y. AYUKAWA, T. SUETSUGU, T. TANAKA (1997): A study of the regional distribution of bone formed around hydroxyapatite implants in the tibia of streptozotocin-induced diabetic rats using multiple fluorescent labeling and confocal laser scanning microscopy. J. Periodontol. 68, 1169-1175.

LANCE, R. M., M. K. STEPHEN, J. SEIYA, M. T. RICHARD, B. JOHN, E. B. MARK (1989): Defects of early fracture healing in experimental diabetes. J. Bone Joint. Surg. 71- A, 722-733.

LEMON, J. E. (1988): Hydroxyapatite coating. Clin. Orthop. 235, 220-223.

PARIZI, A., M. MAMANPOUSH (2003). Bone staple for tibial fracture fixation in young dogs. Indian Vet. J. 80, 144-146.

PALEY, D., M. C. YOUNG, A. M. WIELEY, V. L. FORNASIER, R.W. JACKSON (1986): Percutaneous bone marrow grafting of fractures and bony defects. An experimental study in rabbits. Clin. Ortop. 208, 300-312.

PRAMANIK, S., A. K. AGARWAL, K. N. RAI, A. GARG (2007): Development of high strength hydroxyapatite by solid- state-sintering process. Ceramics Internation. 33, 419-426.

REDDY, K. G., L. STEHNO-BITTEL, S. HAMADA, C. S. ENWEMEKA, (2001): The biomechanical integrity of bone in experimental diabetes. Diab. Res. Clin. Prac. 54, 1-8.

RONALDO, B. S., X. LEI, B. C. HERMIK, A. SALOMON, T. G. DANA, C. T. PHILIP (2003): A role for advanced glycation end products in diminished bone healing in type 1 diabetes. Diabetes 52, 1502-1510.

SEEHERMAN, H. J., K. AZARI, S. BIDIC, L. ROGERS, X. J. LI, J. O. HOLLINGER, J. M. WOZEY (2006): RhBMP-2delivered in a calcium phosphate cement accelerates bridging of critical-sized defects in rabbit radii. J. Bone. Joint. Surg-AM. 88, 1553-1565.

TAKESHITA, F., S. IYAMA, Y. AYUKAWA, M. A. KIDO, K. MURAI, T. SUETSUGU (1997): The effects of diabetes on the interface between hydroxyapatite implants and bone in rat tibia. J. Periodontal. 68, 180-185.

WEISS, R. E., A. H. REDDI (1980): Influence of experimental diabetes and insulin on matrix-induced cartilage and bone differentiation. Am. J. Physiol. 238, 200-207.

WOODARD, J. R., A. J. HILLDORE, S. K. LAN, C. J. PARKB, A. W. MORGAN, J. A. C. EURELL, S. G. CLARK, M. B. WHEELER, R. D. JAMISON, A. J. W. JOHNSON (2007): The mechanical properties and osteocunductivity of hydroxyapatite bone scaffold with multi-scale porosity. Biomaterials. 28, 45-54.

Received: 15 December 2008
Accepted: 22 December 2009

MEIMANDI PARIZI, A., G. JELODAR, H. MOSLEMI, A. K. TAFTI, M. J. EMAMI: Utjecaj hidroksiapatita na cijeljenje prijeloma kostiju dijabetičnih štakora: biomehanička i radiografska istraživanja. Vet. arhiv 80, 113-120, 2010.

SAŽETAK

Pacijenti sa šećernom bolešću često trpe od prijeloma kostiju. To govori da strukturna cjelovitost koštanoga sustava može biti narušena. Ovo istraživanje poduzeto je radi procjene učinaka hidroksiapatita (HA) na cijeljenje prijeloma kostiju u dijabetičnih štakora uporabom biomehaničkih i radiografskih metoda. Ukupno su 24 štakora bila nasumce podijeljena u četiri jednake skupine. U dvjema skupinama dijabetes je bio kemijski potaknut aloksanom. Nakon dva tjedna svi su štakori bili anestezirani te im je pod septičkim uvjetima učinjena transverzalna osteotomija desnog radiusa. Prostor između odlomaka nakon osteotomije štakorima jedne dijabetične i jedne nedijabetične skupine bio je ispunjen hidroksiapatitom dok štakorima drugih dviju skupina hidroksiapatit nije bio primijenjen. Radiografske snimke bile su uzete 0., 15. i 35. dana nakon operacije. Svi su štakori bili eutanazirani pet tjedana nakon operacije te su im uzete radijalne kosti za trotočkasti test savijanja. Usporedba između skupina obrađivanih hidroksiapatitom (dijabetičnih i nedijabetičnih) pokazala je da dijabetes značajno smanjuje sposobnost savijanja kostiju pri opterećenju. Neobrađivana dijabetična skupina pokazivala je manje radiografskih znakova spajanja i ponovnog cijeljenja prijeloma u usporedbi s drugim trima skupinama. Zaključno se može reći da su štakori kojima hidroksiapatit nije bio primijenjen pokazivali slabija biomehanička svojstva i radiografske znakove u usporedbi s onima kojima je bio primijenjen i onima iz kontrolne skupine.

Ključne riječi: hidroksiapatit, cijeljenje prijeloma, dijabetični štakori, biomehanika, radiografija