Short-term alendronate treatment does not maintain a residual effect on spinal fusion with interbody devices and bone graft after treatment withdrawal: an experimental study on spinal fusion in pigs

Baoding Huang · Xuenong Zou · Haisheng Li ·
Qingyun Xue · Cody Büngrer

Received: 23 November 2011 / Revised: 26 July 2012 / Accepted: 9 September 2012 / Published online: 3 October 2012
© Springer-Verlag 2012

Abstract

Purpose Whether alendronate treatment has a residual effect on bone ingrowth into porous biomaterial in humans or experimental animals after treatment withdrawal is still unknown. The purpose of this study was to investigate bone ingrowth into porous tantalum and carbon fiber interbody implants after discontinuing alendronate treatment in experimental spinal fusion in pigs.

Methods Twenty-four pigs were randomly divided into two groups of each 12 pigs. The pigs underwent anterior intervertebral lumbar arthrodeses at L2–3, L4–5 and L6–7. Each level was randomly allocated to one of the three implants: a porous tantalum ring with pedicle screw fixation, a porous tantalum ring or a carbon fiber cage with anterior staple fixation. The central hole of implants was packed with an autograft. Alendronate was given orally for the first 3 months to one of the two groups. The pigs were observed for 6 months postoperatively. Histology and micro-CT scans were done at the endpoint.

Results The spinal fusion rates of each implant showed no differences between two treatment groups. Furthermore, no differences were found between two groups as for bone ingrowth into the central holes of implants and bone–implant interface in each implant, or as for the pores of tantalum implants. Trabecular bone microarchitecture in the central hole of the carbon fiber cage did not differ between two treatment groups.

Conclusion The application of ALN, with a dose equivalent to that given to humans during the first 3 months after surgery, does not maintain a residual effect on spinal fusion with porous tantalum ring and autograft after treatment withdrawal in a porcine ALIF model.

Keywords Alendronate · Residual effect · Tantalum · Pig

Introduction

Alendronate (ALN) sodium is widely used in the management of osteoporosis in postmenopausal women and men. In postmenopausal women, ALN increases bone mineral density, inhibits bone resorption but not bone mineralization, and reduces the incidence of fractures at the spine and hip [1]. ALN showed stimulative [2, 3], inhibitive [4, 5], or no effect [6] on bone fusion/implant osteo-integration. ALN was found to delay maturation and remodeling of fracture callus into lamellar bone [7], and prolonged suppression of bone remodeling after long-time use of ALN may be associated with a new form of atypical fracture [8]. Because of the controversial effect on bone fusion and the inhibitive effect on bone turnover, its influence on relevant biologic processes, such as bone healing, implant integration and so on, received more and more attention.

B. Huang · X. Zou · H. Li · Q. Xue · C. Büngrer
Orthopaedic Research Laboratory, Spine Section/Department
of Orthopaedics E, Aarhus University Hospital,
8000 Aarhus C, Denmark

B. Huang · X. Zou (✉)
Department of Spine Surgery/Orthopaedic Research Institute,
The First Affiliated Hospital of Sun Yat-sen University,
Zhongshan 2nd Road, #58, Building 1, Guangzhou 510080,
Guangdong, People's Republic of China
e-mail: zxnong@hotmail.com

B. Huang
Department of Orthopaedics, The Sixth Affiliated Hospital
of Sun Yat-sen University, Yuan cun Er Heng Road, #26,
Guangzhou 510655, Guangdong, People's Republic of China

Fig. 1 Histomorphometric analysis in four different locations: **a** schematic diagram, central hole of the ring/cage (a zone), pores of porous tantalum (b zone), vertebrae–implant interface (c zone), vertebrae–graft interface (d zone). **b** The sagittal plane of vertebrae–implant specimen which was cut in halves longitudinally through the center of PT-ring

ALN has a high affinity for hydroxyapatite crystals on bone surface, particularly at sites of active bone remodeling, and is deposited in bone where it can exhibit elimination half-life of up to 12 years [9]. Some studies assessed the withdrawal effects of ALN after stopping treatment, and found relatively stable therapeutic effect up to 7 years after ALN treatment withdrawal [10]. With increased life span, more patients with degenerative or traumatic orthopedic conditions are concomitant with osteoporosis in the elderly population. Hence, major concerns have been raised about whether osteoporotic patients, who received ALN treatment, would be impacted on bone integration when undergoing orthopedic implantation.

An implant made of porous biomaterial, such as porous tantalum trabecular metal, offers a viable solution to some challenges in orthopedic reconstructive surgery, including monoblock acetabular cups, spinal interbody cage fusion, and so on. Porous tantalum biomaterials (trade name Hedrocel®) have high porosity, and structural stiffness similar to cancellous bone about 2.5–4.0 GPa. This biomaterial is well-documented as providing favorable tissue ingrowth characteristics in canine transcortical porous tantalum rod and acetabular cups [11, 12]. Bone ingrowth into porous tantalum implants with autogenous bone graft has been characterized in a porcine anterior lumbar interbody fusion model [13, 14].

Our previous study found that continuous treatment after the surgery with daily oral ALN 10 mg for 3 months resulted in increased bone ingrowth into the central hole and the pores of the porous tantalum implant than control without intervention of ALN in anterior lumbar interbody fusion (ALIF) in the porcine model [2], and increased bone ongrowth on the pedicle screw surfaces in posterior lateral spine fusion in pigs [15]. Following aforementioned observations, the aim of the present study was to investigate whether a short-term alendronate treatment maintains a residual effect on anterior lumbar spinal fusion with interbody devices and autograft after treatment withdrawal in pigs.

Animals and methods

Implants

Two types of spinal interbody fusion devices were used, the porous tantalum ring (PT-ring, 23 mm in width, 15 mm in depth, and 9 mm in height, kindly donated by Implex Corp, presently Zimmer Inc., Warsaw, IN, USA) and the Brantigan Cervical I/F carbon fiber cage (CF-cage, 8 mm standard, Depuy Acromed Corp., USA).

Study design

Twenty-four female Danish Landrace pigs about 12 weeks old from different litters, weighing about 50 kg, were randomly assigned to two groups, each for 12 pigs: an experimental group (ALN) given 3-month treatment with ALN (Fosamax, MSD b.v., Denmark) orally in a daily dose of 10 mg from the second day after the operation and discontinuation of ALN treatment for 3-month follow-up, and a control group (control) given no ALN treatment for 6 months. The porcine ALIF model at L2–3, L4–5 and L6–7 was used as before [2, 13, 14]. Each level was randomly allocated to 1 of 3 procedures: (1) implantation of a PT-ring with pedicle screws (Diapason 5 × 30 mm; Stryker® Spine, Cestas, France) fixation (PT-ring + PSF); (2) implantation of a PT-ring with two staples (22 mm in width and 16 mm in depth, Howmedica GmbH, Schönkirchen, Germany) in front of the implant (PT-ring + ASF); (3) implantation of a carbon fiber cage with two staples (CF-cage + ASF). The central hole of implants was packed with iliac crest autograft. The tablet of ALN was given by mixing with diet between two meals so as to get reliable administration of ALN to pigs. The pigs were observed for 6 months before the endpoint. The experiments complied with the Danish Law on Animal Experimentation, and were approved by the Danish Ministry of Justice Ethical Committee, J. no. 1998–561–67.

Table 1 Amount of bone graft

Groups	Amount of graft (g)		
	PT-ring + PSF	PT-ring + ASF	CF-cage + ASF
ALN	0.67 (0.07)	0.68 (0.03)	1.05 (0.03)
Control	0.69 (0.04)	0.68 (0.05)	1.03 (0.03)

The mean values (SD) are shown for the amount of bone graft in the different individual implants in ALN and control groups

Surgical procedure

An autograft was taken from the left iliac crest of the pig under anesthetic aseptic conditions. After ligating and cutting of the segmental vessels, the L2–3, L4–5 and L6–7 intervertebral discs were excised together with the cranial and caudal endplates and part of the anterior longitudinal ligament. The same amount of morselized bone graft was put into the central hole of each implant (Table 1). After insertion, each implant was secured with two staples or pedicle screws. Prophylactic ampicillin and analgesic buprenorphine were given before and immediately after surgery, and twice a day during the following 3 days.

All pigs were kept in the Danish Institute of Agricultural Sciences, Research Center Foulum, and fed with a normal diet containing 1.4 % calcium and 0.7 % phosphorus (percent of food weight). The pigs in control group were kept for mean of 184 days, and the pigs in ALN group were kept for 183 days. The pigs were sacrificed after 6 months.

Specimen preparation

Each vertebrae–implant specimen was cut in halves longitudinally through the center of implants in the sagittal plane (Fig. 1b). One half was randomly chosen for histomorphometric processing. Specimens were dehydrated in graded ethanol (70–99 %) containing 0.4 % basic fuchsin and embedded in methylmethacrylate. 50- μ m-thick sections were cut with 500 μ m intervals between each section. Each was cut to a thickness of 50 μ m, using the sawing microtome KDG 95 (Meprotech, Galileistraat 24, NL-17045E, Heerhugowaard, The Netherlands). The surface was counterstained with 2 % light green for 2 min. Four sections were made from each vertebrae–implant specimen.

Radiography

At the endpoint, the spinal column from L1 to L7 with the sacrum was removed en bloc, stripped of soft tissue. In the same projection of the radiograph, we measured the lengths of the radiolucent lines at both the cranial and caudal vertebrae–implant/graft interfaces, and total lengths of both the cranial and caudal vertebrae–implant/graft interfaces.

The radiolucent percentage was calculated by dividing the length of the radiolucent lines by the total length of the vertebrae–implant/graft interface. The radiolucent lines were categorized according to their relative percentage to represent three different states of healing: A, no radiolucent lines; B, less than 50 % radiolucent lines; or C, more than 50 % radiolucent lines [13].

Histology

All sections were evaluated in blinded manner, and under the light microscope. The pattern of tissue ingrowth into porous tantalum and tissue ongrowth onto carbon fiber cage was classified into three grades. Grade 2 was bone in/ongrowth into the implant material from both vertebral sides with or without inner side having the central hole, and little, if any, partly fibrous tissue surrounding the implant. Grade 1 referred to bone in/ongrowth into the implant material from one vertebral side and/or inner side of central hole of ring/cage, and partly fibrous tissue surrounding the implant. Grade 0 was no bone in/ongrowth into the implant material, and fibrous tissue surrounding the entire implant. A trabecular bridging bone connecting one adjacent vertebral body to the next through the center hole of an implant (appeared on at least one slice) was classified as histological fusion.

Histomorphometry

We used a stereological software program (CAST-Grid, Olympus Denmark A/S, Albertslund, Denmark) for histomorphometry as before [2, 13]. Five different locations on the sections for histomorphometry (a–d zone) are shown in Fig. 1a.

Ingrowth was defined as the central hole (a zone) of the ring/cage and the pore of porous tantalum (b zone) filled by bone, bone marrow or fibrous tissue (as a percentage). About 1,200 points on 4 sections per implant were counted in each region of interest.

Implant-in-bone healing at the vertebrae–implant interface was defined as the region from the implant surface to 2 mm from the surface of bone, bone marrow or fibrous tissue (as a percentage) (c zone). About 500–1,200 points on 4 sections per implant were counted in each region of interest.

Graft-in-bone healing at the vertebrae–graft interface was defined as the region from the graft surface to 2 mm from the surface of bone, bone marrow or fibrous tissue (as a percentage) (d zone). About 600–1,200 points on 4 sections per implant were counted in each region of interest.

Micro-computed tomography (μ -CT) scanning

A high-resolution μ -CT system (μ -CT 40, Scanco Medical AG., Zürich, Switzerland) was used to scan the specimens,

Fig. 2 Posteroanterior and lateral view of three different ALIF in the porcine model. Control group (**a, b**): The CF-cage + ASF (*top*) and the PT-ring + ASF (*bottom*) showed no radiolucent line in PA (**a**) and lateral view (**b**). The PT-ring + PSF (*middle*) showed no radiolucent line in PA view (**a**), but vague radiolucent line in the inferior interface was found in lateral view (**b**). ALN group (**c, d**):

the three ALIF showed no radiolucent line in PA view (**c**). PT-ring + PSF (*top*) or ASF (*middle*) showed minor radiolucent line in the inferior interface, while no radiolucent line either in the central hole or between the host bone and grafted bone (*bottom*) in CF-cage + ASF was found (**d**)

resulting in 3-D reconstruction cubic voxel sizes of $36 \times 36 \times 36 \mu\text{m}^3$. A typical 3-D image data set, which calculated from a cylinder of 5-mm in diameter in the two “outer” zones and one “central” zone, consisted of approximately 240 $\mu\text{-CT}$ slide images in each zone (512×512 pixels) with 16-bit-gray-levels.

The $\mu\text{-CT}$ images were segmented using mean values from optimal thresholds corresponding to Archimedes’-based volume fraction to generate accurate 3-D data sets [16]. With these 3D images, assessment of morphometric parameters in the central hole of CF-cage, including bone volume (BV), bone volume fraction (percentage of bone voxels per total specimen voxels, BV/TV), mean trabecular thickness based on 3-D analysis in μm (TbTh*) (asterisk indicates true 3-D measure), bone surface-to-volume ratio in mm^{-1} (BS/BV), and structural model index (SMI) was performed using direct distance transformation methods. Unbiased assumption-free 3-D connectivity (CD), number of trabeculae per volume in mm^3 (TbN*), was quantified topologically. Bone surface density in mm^{-1} (BS/TV) and 3-D trabecular spacing in μm (TbSp*) were calculated.

Statistical analysis

The Chi-square test was used for nonparametric values. The statistical analyses of parametric data included two-way analyses of variance (ANOVA) for repeated measures of the tissue volume fraction. The group (ALN vs. control) was used as the between-subjects factor and procedures (PT-ring + PSF, PT-ring + ASF, and CF-cage + ASF) as

the within-subjects factor. When significant main effects or an interaction between the main effects was found, specific comparisons were made with student’s *t* tests and paired *t* tests. In all cases, exact *p* values were given and we considered $p < 0.05$ to represent significant effects. Statistical analysis was done with SPSS version 10.0 statistical software (SPSS, Chicago, IL, USA).

Results

Surgery

All pigs survived the surgical procedure. One pig from control group was terminated due to early infection after surgery, and one pig from ALN group was excluded from the analysis due to infection at a late stage of the observation period.

Radiographic examination

No obvious kyphosis, migration or fracture of interbody implants was observed on radiographic examination after 6 months. No bone resorption was seen around the pedicle screws. Although it was impossible to evaluate by plain radiographs whether grafted bone inside a PT-ring was fused, radiolucencies could be clearly seen when they were present between implant and adjacent vertebral bodies (Fig. 2d). Representative radiographs showed the posteroanterior (AP) and lateral views of specimens from control and ALN group (Fig. 2).

Fig. 3 Histological examination of specimen sections showed that the CF-cage + ASF had dense trabecular bone through the center of the cage, with fibrous tissue surrounding most of the CF-cage and sometimes extended across the central hole of the device, preventing bridging bone (**a, d**). Well-organized trabecular bone oriented longitudinally through the central hole of the PT-ring and trabecular bone ingrowth into the porous tantalum from all directions with junction healing at both cranial and caudal implant interface (**b, e, f**), or at cranial implant interface (**c**)

Table 2 Bone in/ongrowth into three implants

Implantations	n	ALN group (grades)			Control group (grades)			p values
		0	1	2	0	1	2	
PT-ring + PSF	22	0	1	10	0	3	8	0.6
PT-ring + ASF	22	1	5	5	1	5	5	1.0
CF-cage + ASF	22	4	3	4	5	3	3	1.0

Histological examination

Histological results showed that well-organized trabecular bone oriented longitudinally through the central hole of the PT-Ring and trabecular bone ingrowth into the porous tantalum from all directions with junction healing at both cranial and caudal implant interface in the most of specimens (Fig. 3b, c, e, f). The CF-Cage + ASF had dense trabecular bone through the center of the cage, with fibrous tissue surrounding most of the CF-cage and sometimes extended across the central hole of the device, preventing bridging bone (Fig. 3a, d). The pattern of tissue ingrowth into the PT-ring and of tissue ongrowth onto the CF-cage was similar in each device in the ALN-treated pigs and controls (Table 2).

Histological fusion with continuous trabecular bridging across the central holes of implants was 91 % (10/11) in the PT-ring + PSF in both groups. However, the fusion rate was only 56 % (6/11) in the PT-ring + ASF in both groups, and 56 % (6/11) in the CF-cage + ASF in the ALN-treated pigs and 64 % (7/11) in the controls. The rate of spinal fusion was not different in each device in the ALN-treated pigs and controls (Table 3). Representative

Table 3 Spinal fusion rates in three implants

Implantations	n	ALN group		Control group		p values
		Fusion (%)	Non-fusion (%)	Fusion (%)	Non-fusion (%)	
PT-ring + PSF	22	10 (91)	1 (9)	10 (91)	1 (9)	1.0
PT-ring + ASF	22	6 (56)	5 (44)	6 (56)	5 (44)	1.0
CF-cage + ASF	22	6 (56)	5 (44)	7 (64)	4 (36)	1.0

pictures showed fused and non-fused PT-ring and CF-Cage (Fig. 4).

Histomorphometry

Tissue ingrowth The bone and fibrous tissue ingrowth into the central hole and the pores were not different in the ALN-treated group compared to controls (Table 4). Although the analysis of variance for the bone and fibrous tissue ingrowth indicated that neither the group (ALN-treated pigs and controls) nor the interaction between the implants and group was significant, the bone and fibrous tissue ingrowth into the various implants was significant in the central hole ($p < 0.001$, as regards the within-subject effect in the analysis of variance). The fibrous tissue ingrowth into the pores of tantalum was greater in PT-ring + ASF than in PT-ring + PSF ($p = 0.003$, as regards the within-subject effect in the analysis of variance), but bone ingrowth into the pores of tantalum was not significant (Table 4).

Implant-in-bone healing The analysis of variance for the volume fraction of the bone and fibrous tissue at the vertebrae-implant interface indicated that neither the group

Fig. 4 Histological results showed fused PT-ring with longitudinally oriented trabecular bone through the central hole, trabecular bone ingrowth into the pores of the implant, and superior and inferior interface healing (a). Fused CF-cage with dense trabecular bone through the central hole, less surrounding fibrous tissue (b). Non-fused PT-ring with fibrous tissue surrounding the implant, ingrowth into the pores, and extended across the central hole (c). Non-fused CF-cage with thicker fibrous tissue around and extending to the central hole of the device (d)

Fused

Not fused

Table 4 Tissue ingrowth into the central hole and the pores of implants 6 months postoperatively

Ingrowth	ALN group			Control group		
	PT-ring + PSF	PT-ring + ASF	CF-cage + ASF	PM-ring + PSF	PM-ring + ASF	CF-cage + ASF
Central hole (%)						
Bone	26 (8)	35 (8)	45 (7)	25 (10)	38 (11)	46 (7)
Fibrous tissue	1 (2)	9 (14)	17 (13)	2 (5)	7 (7)	17 (9)
Pores (%)						
Bone	20 (5)	19 (10)	16 (6)	16 (4)		
Fibrous tissue	33 (17)	54 (23)	42 (20)	63 (14)		

The mean values (SD) are shown for bone and fibrous tissue in the various implants in ALN and control groups

nor the interaction between implants and group was significant (Table 5). The volume fraction of fibrous tissue at the vertebrae–implant interface was greater in CF-cage than in PT-ring ($p = 0.005$, concerning the within-subject effect in the analysis of variance), but it was not significant for the volume fraction of the bone at the vertebrae–implant interface (Table 5).

Graft-in-bone healing We found no change in the volume fraction of bone and fibrous tissue in the regions of the vertebrae–graft interface in the ALN-treated group, as compared to the control group, and no significant interaction between implants and the group (Table 5). The analysis of variance for the volume fraction of fibrous tissue in the regions of the vertebrae–graft interface indicated that it was greater in PT-ring + ASF than in PT-ring + PSF or CF-cage + ASF ($p = 0.001$, as involving the within-subject effect in the analysis of variance), but it was not significant for the volume fraction of the bone in the regions of the vertebrae–graft interface in the various implants (Table 5).

μ -CT scanning

Due to the metal artifacts caused by PT-ring, we only detected the bone properties in CF-Cage-treated vertebrae with μ -CT (Fig. 5a). Representative μ -CT images are shown in Fig. 5b, c. The main morphometric parameters in the central hole of CF-cage of μ -CT images reflecting microstructural properties of cancellous bone, including BV/TV, TbTh*, BS/BV, TbN*, SMI, CD, BS/TV, and TbSp* were the same between alendronate-treated pigs and controls (Table 6).

Discussion

Although ALN was reported to inhibit or delay spinal fusion through reduced incorporation between grafted bone and host bone [4, 5], it was also found to be beneficial for spine fusion in animal studies and clinical trials [2, 3, 17].

Table 5 Implant-in-bone healing and graft-in-bone healing at vertebrae-implant interface 6 months postoperatively

	ALN group			Control group		
	PT-ring + PSF	PT-ring + ASF	CF-cage + ASF	PT-ring + PSF	PT-ring + ASF	CF-cage + ASF
Implant-in-bone healing (%)						
Bone	35 (3)	36 (5)	39 (5)	36 (3)	38 (5)	37 (6)
Fibrous tissue	2 (4)	12 (8)	17 (7)	1 (2)	11 (7)	20 (9)
Graft-in-bone healing (%)						
Bone	31 (4)	32 (7)	40 (5)	33 (5)	36 (10)	42 (5)
Fibrous tissue	1 (3)	11 (12)	2 (3)	0.2 (0.4)	9 (13)	4 (5)

The mean values (SD) are shown for bone and fibrous tissue in the various implants in ALN and control groups

Fig. 5 μ -CT scanning of the CF-cage. The sagittal plane of vertebrae-CF-cage cut in halves longitudinally (a). The sagittal plane of fused CF-cage (b), and non-fused CF-cage (c)

The high affinity to actively remodeling bone, and the residual effect caused by long elimination time raised concerns about whether ALN affects bone integration after ceasing treatment following orthopedic surgery. ALN sustained the bone mass and trabecular network around the titanium implants after its withdrawal in rats [18]. The withdrawal of ALN after 8 weeks showed better subchondral bone repair, and avoided a delayed bone remodeling in continuous use of ALN for 24 weeks [19]. Our previous study showed that 3-month ALN treatment after the surgery increased bone ingrowth into the central hole and the pores of the porous tantalum implant in the porcine model [2]. However, no data was reported about whether ALN treatment has a residual effect on bone ingrowth into porous biomaterial in humans or experimental animals after treatment withdrawal. The present study showed no difference in each device on bone in/ongrowth, in spinal fusion rates, and in fibrous tissue ingrowth between ALN-treated pigs and controls detected by histological and histomorphometric examination, and μ -CT. These results suggested ALN treatment for 3 months did not maintain a residual effect on spinal fusion, or interfere with bone maturation and remodeling in tantalum ring after treatment withdrawal in pigs.

The histologic results showed that the healing parameters of pedicle screw fixated implants were better than that of staple-secured implants in an anterior interbody fusion in pigs. The histomorphometric results showed that the fibrous tissue formation in the central holes, tantalum

pores, vertebral-implant and vertebral-graft interface in PT-ring + PSF was less than PT-ring + ASF. The fibrous tissue formation in the central holes and vertebral-implant interface in PT-ring + ASF was less than CF-cage + ASF. These findings could be explained from the different type of supplementary fixation, different designs of two cages, and the nature of these two biomaterials. The pedicle screw instrumentation provided more rigid fixation for immediate stabilization [20], thus reduced the fibrous formation. The high porosity and structural stiffness of porous tantalum similar to cancellous bone make PT-ring more beneficial for the bone graft incorporation and remodeling than the CF-cage composed of carbon fiber which has structural stiffness similar to cortical bone [11, 13]. Furthermore, tantalum could stimulate cell proliferation, and improve the osteogenesis of human osteoblasts [21]. Alendronate did not inhibit the formation of fibrous tissue in our model [2]. Hence, it is essential to maintain the initial stability with rigid fixation to achieve better fusion.

There are some limitations in the present study. First, although 2 months of follow-up studies witnessed the significant effects of ALN on spine fusion in rabbits and rats [3–5], and 3 months of follow-up studies witnessed increased bone ingrowth into the porous tantalum ring and pores in the same setting as in the present study [2], it would be helpful to have a concurrent group with a continuous treatment for 6 months. Second, the bone remodeling is rapid in growing pigs. The time required for the formation and reconstruction of a complete bone structural

Table 6 Bone ingrowth into the central hole of the carbon fiber cage 6 months postoperatively

	ALN group	Control group
BS	2301.47 (89.18)	2526.17 (118.16)
BV	267.79 (19.38)	300.95 (15.98)
TV	545.02 (17.86)	600.20 (15.73) ^a
BS/BV	9.06 (0.70)	8.66 (0.63)
BS/TV	4.23 (0.13)	4.20 (0.14)
BV/TV	49.00 (3.00)	50.00 (3.00)
SMI	−1.17 (0.48)	−1.48 (0.48)
P-ratio	1.44 (0.06)	1.47 (0.07)
TbTh*	0.25 (0.01)	0.25 (0.01)
TbSp*	0.43 (0.03)	0.39 (0.02)
TbN*	2.50 (0.09)	2.54 (0.10)
CD	17.00 (1.52)	14.70 (1.47)

The mean values (SD) are shown for 3D bone structures in ALN and control groups

^a $p < 0.05$ compare to ALN group

unit, which marks the termination of bone remodeling, is between 3 and 5 months in humans compared to 20 days in immature pigs [22]. ALN treatment for 3 months in the present study is still a relatively short time in contrast to continuous medication for several years in osteoporotic patients; immature pigs receiving ALN treatment for 3 months seem to be equivalent to human beings that receiving continuous medication for one and half years [22]. Finally, although the present study produced information regarding the residual effect of alendronate on spinal fusion, it cannot be totally applied to patients already under treatment with ALN prior to surgery, which is more likely to be major clinical cases.

In conclusion, the application of ALN, with a dose equivalent to that given to humans during the first 3 months after surgery, does not maintain a residual effect on spinal fusion with porous tantalum ring and autograft after treatment withdrawal in a porcine ALIF model in the present study. To our knowledge, this is the first study of the residual effect of ALN withdrawal on spinal fusion. Because the patients usually have taken ALN for a long time before the surgery, further research is needed to detect residual effect on spinal fusion after long-term use of ALN with the experimental design more close to clinical condition.

Acknowledgments The authors thank Anette Milton for technical assistance in histology, Bruce Robie, PhD, for excellent help in revising of the manuscript, and the Danish Institute of Agricultural Sciences, Research Center Foulum, for excellent cooperation. The Danish Rheumatism Association, the Institute of Experimental Clinical Research, University of Aarhus, and the Aarhus Spine Research Foundation in Denmark provided financial supports. This work was

also supported by the Danish Council for Strategic Research project IMR: “Individualized musculoskeletal regeneration and reconstruction” (2101-07-0120), 973 Program (No. 2012CB619100), the NSFC (No. u0732001), and the S&T project of Guangdong Province (2010B010800019). Hedrocel ALIF devices (Porous Tantalum Implants) was kindly supplied by Implex Corp., Allendale, NJ, USA, for the study. The experiments complied with the Danish Law on Animal Experiments and were approved by the Danish Ministry of Justice, J. No. 1998-561-67.

Conflict of interest None.

References

1. Liberman UA, Weiss SR, Broll J et al (1995) Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate phase III osteoporosis treatment study group. *N Engl J Med* 333:1437–1443
2. Zou X, Xue Q, Li H et al (2003) Effect of alendronate on bone ingrowth into porous tantalum and carbon fiber interbody devices: an experimental study on spinal fusion in pigs. *Acta Orthop Scand* 74:596–603
3. Nakao SI, Minamide A, Kawakami M et al (2011) The influence of alendronate on spine fusion in an osteoporotic animal model. *Spine (Phila Pa 1976)* 36:1446–1452
4. Huang RC, Khan SN, Sandhu HS et al (2005) Alendronate inhibits spine fusion in a rat model. *Spine (Phila Pa 1976)* 30:2516–2522
5. Lehman RA Jr, Kuklo TR, Freedman BA et al (2004) The effect of alendronate sodium on spinal fusion: a rabbit model. *Spine J* 4:36–43
6. Sama AA, Khan SN, Myers ER et al (2004) High-dose alendronate uncouples osteoclast and osteoblast function: a study in a rat spine pseudarthrosis model. *Clin Orthop Relat Res* 425: 135–142
7. Peter CP, Cook WO, Nunamaker DM et al (1996) Effect of alendronate on fracture healing and bone remodeling in dogs. *J Orthop Res* 14:74–79
8. Lenart BA, Lorich DG, Lane JM (2008) Atypical fractures of the femoral diaphysis in postmenopausal women taking alendronate. *N Engl J Med* 358:1304–1306
9. Lin JH, Russell G, Gertz B (1999) Pharmacokinetics of alendronate: an overview. *Int J Clin Pract Suppl* 101:18–26
10. Bagger YZ, Tanko LB, Alexandersen P et al (2003) Alendronate has a residual effect on bone mass in postmenopausal Danish women up to 7 years after treatment withdrawal. *Bone* 33:301–307
11. Bobyn JD, Stackpool GJ, Hacking SA et al (1999) Characteristics of bone ingrowth and interface mechanics of a new porous tantalum biomaterial. *J Bone Joint Surg Br* 81:907–914
12. Bobyn JD, Toh KK, Hacking SA et al (1999) Tissue response to porous tantalum acetabular cups: a canine model. *J Arthroplasty* 14:347–354
13. Zou X, Li H, Bünger M et al (2004) Bone ingrowth characteristics of porous tantalum and carbon fiber interbody devices: an experimental study in pigs. *Spine J* 4:99–105
14. Bünger MH, Foss M, Erlacher K et al (2006) Bone nanostructure near titanium and porous tantalum implants studied by scanning small angle X-ray scattering. *Eur Cell Mater* 12:81–91
15. Xue Q, Li H, Zou X et al (2010) Alendronate treatment improves bone-pedicle screw interface fixation in posterior lateral spine

- fusion: an experimental study in a porcine model. *Int Orthop* 34:447–451
16. Ding M, Odgaard A, Hvid I (1999) Accuracy of cancellous bone volume fraction measured by micro-CT scanning. *J Biomech* 32:323–326
 17. Nagahama K, Kanayama M, Togawa D et al (2011) Does alendronate disturb the healing process of posterior lumbar interbody fusion? A prospective randomized trial. *J Neurosurg Spine* 14:500–507
 18. Duarte PM, de Vasconcelos Gurgel BC, Sallum AW et al (2005) Alendronate therapy may be effective in the prevention of bone loss around titanium implants inserted in estrogen-deficient rats. *J Periodontol* 76:107–114
 19. Nishitani K, Shirai T, Kobayashi M et al (2009) Positive effect of alendronate on subchondral bone healing and subsequent cartilage repair in a rabbit osteochondral defect model. *Am J Sports Med* 37:139S–147S
 20. Lund T, Oxland TR, Jost B et al (1998) Interbody cage stabilization in the lumbar spine: biomechanical evaluation of cage design, posterior instrumentation and bone density. *J Bone Joint Surg Br* 80:351–359
 21. Sagomonyants KB, Hakim-Zargar M, Jhaveri A et al (2011) Porous tantalum stimulates the proliferation and osteogenesis of osteoblasts from elderly female patients. *J Orthop Res* 29(4):609–616
 22. Schenk RK, Felix R, Hofstetter W (1993) Morphology of connective tissue: bone. In Royce, PM, Steinmann B (eds) *Connective Tissue and Its Heritable Disorders. Molecular, genetic, and medical aspects*, Wiley-Liss, New York, pp 85–101