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Healing properties of allograft from alendronate-treated animal in lumbar spine interbody cage fusion

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Abstract This study investigated the healing potential of allograft from bisphosphonate-treated animals in anterior lumbar spine interbody fusion. Three levels of anterior lumbar interbody fusion with Brantigan cages were performed in two groups of five landrace pigs. Empty Brantigan cages or cages filled with either autograft or allograft were located randomly at different levels. The allograft materials for the treatment group were taken from the pigs that had been fed with alendronate, 10 mg daily for 3 months. The histological fusion rate was 2/5 in alendronate-treated allograft and 3/5 in non-treated allograft. The mean bone volume was 39% and 37.2% in alendronate-treated or non-treated allograft (NS), respectively. No statistical difference was found between the same grafted cage comparing two groups. The histological fusion rate was 7/10 in all autograft cage levels and 5/10 in

combined allograft cage levels. No fusion was found at all in empty cage levels. With the numbers available, no statistically significant difference was found in histological fusion between autograft and allograft applications. There was a significant difference of mean bone volume between autograft (49.2%) and empty cage (27.5%) ($P < 0.01$). In conclusion, this study did not demonstrate different healing properties of alendronate-treated and non-treated allograft for anterior lumbar interbody fusion in pigs.

Keywords Anterior lumbar interbody fusion (ALIF) · Bisphosphonate · Bone graft · Cage · CT

Introduction

Bisphosphonates have been widely used for treatment of osteoporosis. The biological effects of bisphosphonates on calcium metabolism have been ascribed to their physico-chemical effects on hydroxyapatite crystals and action on cells [7]. Osteoclast activity is important in bone graft healing and incorporation. Bisphosphonates have been reported to modulate the remodeling phase of ovine bone in fracture repair, resulting in larger and stronger callus tissue [13]. Allograft immersed in a bisphosphonate solution before

implantation could protect allograft from resorption [3]. Some bisphosphonate-treated patients may have to undergo orthopedic surgery and/or serve as an allograft donor. Alendronate pretreatment may alter bone-graft-healing behavior. The healing capability of bone graft from bisphosphonate-treated individuals is insufficiently understood.

The hypothesis of this study is that alendronate-treated allograft has equal healing properties compared with non-treated allograft for anterior lumbar interbody fusion. The aim of this study was to investigate the behavior of alendronate-treated allograft and to compare it with allograft and autograft in anterior lumbar spine interbody fusion.

Materials and methods

A porcine model of an anterior lumbar interbody fusion was used in this study [16]. Two groups of five female landrace pigs, 3 months old and about 50 kg each, were selected. Three levels of anterior lumbar interbody fusion (at spinal levels L2/3, L4/5, L6/7) with Brantigan I/F cages (8 mm; AcroMed, Cleveland, OH, USA) were performed on each pig in a randomized design. At one level, a Brantigan cage was filled with autograft. At another level, the cage was filled with the same amount of allograft bone. Each implanted cage was weighed before and immediately after packing the bone graft into its central cavity. The weight of bone graft in each cage was uniformly kept at 1 g. Allograft material used for five pigs in the treatment group was taken from five female landrace pigs that were fed with alendronate, 10 mg daily per os for 3 months. In the third level, an empty cage was employed. The spinal levels for each of the three applications were randomly selected. The experiment was approved by the state inspection for animal experiments (1998–561–67-CBSC 01101).

Bone graft preparation

Autograft material was taken from the right iliac crest during the operation on each pig. Under sterile conditions, allograft materials were harvested from the iliac crests of two groups of landrace pigs, which have been used in another study and were 6 months old. In one of these two groups, the pigs were fed with alendronate 10 mg/daily per os for the last 3 months before bone graft was harvested. The allograft materials were washed in saline and kept deep frozen (-80°C) in a sterilized manner. During operation, the allograft material was thawed at room temperature and cut into pieces. All the cancellous bone graft materials were cut manually into pieces about 2–3 mm in size.

Surgical procedure

The anterior L2/3, L4/5 and L6/7 discectomies and interbody fusions were performed through a vertical paramedian retroperitoneal approach on the left side of the abdomen. The L2/3, L4/5 and L6/7 disc spaces were exposed adequately. The disc tissue and the endplates of both vertebral bodies adjacent to the disc were removed by bone chisel. The template spacer was applied and followed with the placement of a Brantigan cage. Two titanium alloy staples (22×16; Howmedica, Schönkirchen, Germany) were placed over the operated disc spaces to enhance stabilization. When the three levels of interbody fusion with cage had been accomplished, the incision was closed anatomically. Prophylactic ampicillin (1.0 g, I.V. Anhyphen; Gist-Brocades, Delft, The Netherlands) was given before and immediately after surgery and analgesic buprenorphine (Temgesic; Hull, UK, 0.3 mg, I.M.) was given postoperatively twice a day for 3 days. The pigs were kept in individual pens and allowed free activity.

Specimen preparation

Each animal was killed at 12 weeks postoperatively by intravenous injection of overdose pentobarbital. The spinal segment from L1 to L7 was removed en bloc. The specimens were kept deep frozen (-20°C) prior to the examination process.

Plain anterior–posterior and lateral radiographs as well as computer tomography of the spine were obtained. CT scans (MX 8000 High Speed Scanner, Marconi, USA) of 2-mm-thick slices with 1-mm increments in transverse and sagittal planes were made (Fig. 1). Multiple slices were reviewed for fusion mass on the sagittal planes.

Two individuals under blind circumstances evaluated grade of fusion on X-rays and CT scans. A continuous bony bridge crossing

Fig. 1 X-ray and CT images at 3 months after operation. From top to the bottom, first level: an empty cage; second level: a cage filled with autograft; third level: a cage filled with allograft

through the central cavity of Brantigan cage was considered to constitute fusion.

Histomorphometry

The fusion masses were harvested together with a sufficient amount of neighboring vertebral body tissue. They were dehydrated in graded ethanol (70–99%) containing 0.4% basic fuchsin and embedded in methyl methacrylate. Each fusion mass was cut along the central line through the sagittal plane. Either the left part or right part was selected randomly for histomorphometric examination. Serial sections were cut to obtain sagittal sections. In order to obtain unbiased estimates, the second section (on average, four sections per-level) with a random start was used for histomorphometric evaluation. Each was cut to a thickness of 50 μm using the microtome KDG 95 (MeProtech, The Netherlands). The surface was counterstained with 4% light green for 1 min. Blinded quantitative evaluation of bone volume was performed using the point-counting technique (CAST-Grid software, Olympus, Glostrup, Denmark). In order to obtain random samples, a standard counting frame was overlaid on the sections under the light microscope. The box was moved from left to right and right to left, in turn, for each row. Four slices and, on average, 1,710 points were counted at each level. Bone volume with the bone marrow and fibro-tissue volumes were calculated in percentage for each fusion mass. A trabecular bridging bone connecting one adjacent vertebral body to the next through the center cavity of a Brantigan cage (appeared on at least one slice) was classified as histological fusion. The fusion rates and bone volumes of the various grafting groups were compared.

Statistics

One-way analyses of variance (ANOVA) were used to analyze difference of bone volume between autograft, allograft and empty cage ($n=10$). When comparing two groups, the sample size was small ($n=5$) for each kind of bone graft.

A Mann–Whitney test for nonparametric data was used. The results are given as mean. A 5% two-tailed limit of statistical significance was used for all calculations. SPSS 10.0 was the statistical software used.

Results

The histological fusion rate was 2/5 in alendronate-treated allograft and 3/5 in non-treated allograft. The mean bone volume was $39.0\% \pm 7.8\%$ and $37.2\% \pm 2.8\%$ in alendronate-treated or non-treated allograft. There was no statistically significant difference in mean bone volume or fusion rate between treatment and control groups.

Compared within groups, a statistically significant difference of bone volume was only found between the autografted cages and empty cages ($P=0.028$ in control group and $P=0.016$ in treatment group, Mann–Whitney test). When the same grafted-cage data from two groups was pooled, the histological fusion rates for autograft and the combined allograft-cage levels were 70% (7/10) and 50% (5/10), respectively. No bony fusion was found at the empty cage levels (0/10). Cross-tabulation analysis showed a significant difference in fusion rates between autografted and empty cages ($P<0.01$) and between allografted and empty cages ($P<0.05$). The difference in fusion rate between autograft and allograft cages was not statistically significant ($P=0.65$). The mean bone volume was $49.2\% \pm 2.9\%$ at autograft levels and $37.8\% \pm 4.4\%$ at allograft levels, and $27.5\% \pm 3.5\%$ at empty cage levels. There was significant difference in mean bone volumes between autograft and empty cages ($P<0.01$, Bonferroni correction). No statistical difference was found between autograft and allograft cages ($P=0.16$, Bonferroni) or between allograft

Table 1 Tissue volumes and fusion rates of various grafted cages. Data are expressed as the mean $\pm$ SEM

Graft	Bone volume%	Bone marrow volume%	Fibrous tissue volume%	Histological fusion%
Empty	27.45 ± 3.46	18.4 ± 1.75	$54 \pm 4.92\#$	0/10
Autograft	$49.2 \pm 2.88\%$	25 ± 1.79	25.6 ± 3.57	7/10**
Allograft	37.8 ± 4.44	23.7 ± 3.52	37.5 ± 7.64	5/10*

* $P<0.05$ Fusion rate between allograft and empty cage

** $P<0.01$ Fusion rate between autograft, and empty cage

§ $P<0.01$ Bone volume between autograft and empty cage

$P<0.01$ Fibrous tissue volume between autograft and empty cage

and empty cages in mean bone volumes ($P=0.11$, Bonferroni). The histological fusion rate and bone volume evaluation under light microscope are shown in Table 1. At non-fusion level, the fibrous tissue often constituted a dense, predominant band crossing through the transverse plane in the central cavity of the Brantigan cage. At the empty cage level, the central part of the cage cavity was filled out by a mass of fibrous tissue. The fibrous tissue volume was $54\% \pm 4.9\%$ at the empty cage levels. Some bone had grown into the central cavity of the cage from two adjacent vertebral bodies (Fig. 2a, b, c).

As the Brantigan cage is radiotransparent, bone fusion in the central cavity of the cage could be identified. If radiolucent lines appeared at the central part or at either end of a Brantigan cage, it was considered a non-fusion. The fusion rate based on X-ray evaluation was 4/10 at autograft levels and 2/10 at allograft levels. Four of 8 autograft levels and 1/8 allograft levels were considered fused based on CT scans (two cases were missed in CT image diagnosis due to digital data loss). No fusion was found at empty cage levels.

Discussion

Bisphosphonates have a high affinity for deposit to bone structure. The oral bioavailability of alendronate in the fasted state is about 0.7%. About 40–60% of the alendronate will deposit to bone [18]. Bisphosphonates bind to bone surfaces and subsequently inhibit osteoclast activ-

Fig. 2 **a** A cage without bone graft had a mass of fibrous tissue inside the cage; **b** a cage with autograft had a solid bone fusion; **c** a cage with allograft also had bony fusion

ity, slowing down bone-turnover rate. In a bone chamber study, the local use of alendronate has been shown to protect the allograft from resorption [3]. Treatment with bisphosphonate provides a means to reduce bone resorption [7, 15, 21]. In the present study, allograft materials taken from alendronate-treated pigs were used in five cages. A daily per os dosage of 10 mg alendronate, which is the common dosage for humans, was administered, as the animal chosen for our research is, in relative size and body weight, comparable to humans. During the 3-month treatment, the bisphosphonate could deposit into the bone tissue. This may alter the healing process and graft incorporation of the allograft. The role of alendronate-treated bone graft resorption is not yet clear. The present study did not demonstrate statistical difference between the two kinds of allografts. A tendency was found for the alendronate-treated allografts to have higher bone volume. The bone graft in our study seems to have had a lower concentration of alendronate, but it had a more "open" environment than that in Aspenberg's bone chamber study [3]. This difference in environment could have reduced bisphosphonates' effect of inhibiting resorption. The concentration of bisphosphonate in bone was found to correlate closely with the given dose, treatment modes and turnover rate of bone tissue. Without continuous bisphosphonate administration, the pre-loaded alendronate will either be covered by newly formed bone or be washed out during the bone-graft remodeling process. Our data from the present study show no clear evidence of reduced bone-graft incorporation due to previous alendronate treatment. This was in agreement with the findings in Li et al.'s study, which suggested that discontinuing bisphosphonate treatment was beneficial for the recovery of remodeling in the bone healing process [17]. In our study, previous treatment with alendronate seems risk-free for allograft donation.

Autograft is ideal material for enhancing spinal fusion. It has osteogenic, osteoinductive and osteoconductive properties [5]. However, extracting bone from an iliac crest for grafting involves an additional surgical trauma and possible donor site complications for the patient [2, 4]. Using allograft bone is associated with a small risk of infectious disease transmission, but it will eliminate the need for harvesting iliac crest bone and its associated morbidity [11]. Allografts are mainly osteoconductive owing to a lack of living cells and a diminished biochemical activity of the bone tissue. They undergo stages of incorporation similar to those of autologous bone [5]. Allografts can in-

voke immunologic inflammatory responses during the bone graft incorporation. This can result in extensive resorption of the graft while bone is being formed in the healing process [19]. The role of bone graft resorption is not yet clear, but bone-graft resorption reduces the progenitive basis for new bone formation, which could be a factor underlying the failure of spine fusion.

Several studies have reported using allograft in spinal fusion enhancement [6, 14] and comparing the use of autograft vs allograft in spine fusion. Some authors found equal fusion rates [9, 10, 12], and others found an inferiority for allograft if compared with autograft [1, 8]. In the present study, both autograft and allograft underwent an incorporation process, as evidenced by histological examination. Seven of 10 of the autograft cages and only five of 10 of the allograft cages presented histological fusion 3 months, post-operatively; i.e., equal amounts of graft materials resulted in 20% less fusion rate and 11.4% less bone volume for the allograft group compared with the autograft group.

Non-fusion was found at all empty cage levels. Histological evaluation revealed the central part of the cage cavity to be occupied by a mass of fibrous tissue. Although the empty cage had a 27.5% mean bone volume, most of the newly formed bone was located adjacent to a neighboring vertebral body. The results indicate that bone graft is needed for induction of bone growth and, thereby, the preclusion of fibrous tissue formation inside the cage. These findings are consistent with other studies indicating that osteoconductive material, such as bone graft, can increase growth [20].

This study's limitations include limited sample size, which may reduce clinical relevance, and skeletally immature animals that may have higher individual sample variation. We realize that it is unknown exactly how much alendronate was deposited on bone during the 3-month treatment in this pig model. We intend to examine this in a future study.

Conclusion

Allograft from alendronate-treated animals did not show different healing qualities from non-treated allograft in this study.

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