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The influence of intervertebral disc tissue on anterior spinal interbody fusion: an experimental study on pigs

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Abstract Intervertebral disc has been shown to be related to low back pain and nerve root injury in pathologic conditions. However, little is known about its influence on spinal fusion. With the development of minimal invasive operations, such as laparoscopic anterior spinal fusion with cages, insufficient discectomy may occur. With its inflammatory properties, the residue nucleus pulposus may have an effect on spinal fusion. In this study, a two-level lumbar spine interbody fusion (L3/4, L5/6) with a Brantigan cage was performed on ten Danish Landrace pigs. Each level was randomly assigned to one of the following methods: (1) implantation of Brantigan cage filled with autogenous iliac crest bone graft, or (2) implantation of Brantigan cage filled with a mixture of autograft and the nucleus pulposus tissue harvested from the disc level in which it was to be inserted. Each level was stabilized with two staples. The pigs were followed for 12 weeks in the same standardized condition. After sacrifice, the lumbar spines

were taken out, and plain X-ray, computed tomographic (CT) scanning and histomorphometry were performed to study the fusion mass inside the cages. From plain radiographs, new bone formation could be seen inside and around the cage. CT evaluation showed that the nucleus pulposus level had a 20% (2/10) fusion rate, while the pure autograft level had a 70% (7/10) fusion rate ($P=0.07$). The histological fusion rate was even lower in the nucleus pulposus level (10%), and was significantly different from the autograft level (70%, $P=0.02$). Histomorphometric parameters of new bone formation, bone marrow space and fibrous tissue differed significantly between the two levels ($P=0.04$; $P=0.02$; $P=0.04$ respectively). We conclude that when nucleus pulposus is mixed with the autogenous bone graft, it can delay or decrease the bone formation inside the cage, thus influencing the final fusion.

Keywords Spine fusion · Interbody fusion · Nucleus pulposus · Pig

Introduction

Interbody spinal fusion has been performed extensively in clinical practice. The excision of the intervertebral disc is a standardized procedure. However, with the development of minimal invasive operations, such as laparoscopic anterior spinal fusion with cages and posterior

lumbar interbody fusion (PLIF), insufficient discectomy can occur in some cases. In a comparative study of laparoscopic lumbar fusion and open operations in pigs, Riley et al. [15] found a less extensive discectomy and less bone formation in the implants of the laparoscopic group.

As one of the disc components, nucleus pulposus (NP) has attracted attention due to its direct relationship to lower

back pain and nerve root injury under pathological circumstances [1, 8]. Little is known, however, about its influence on spinal fusion. Disc tissue, especially NP, has been reported to possess inflammatory properties [7, 4], and can secrete cytokines [14] that are closely related to the metabolism of osteoblast [17]. Furthermore, exposure of NP, which is normally excluded from circulation, to the fusion environment may also cause some immune reactions [4, 7, 13]. Our previous *in vitro* study showed that disc tissue could influence the metabolism of osteoblast-like cells [9]. In view of the difficulties of evaluating cage fusions clinically, and given a lack of randomised controlled studies with long-term follow-ups as well as sufficiently documented outcomes of spinal fusion rates from minimal invasive surgeries, it was deemed necessary to clarify this issue by conducting an animal experiment. Accordingly, the present study was designed to investigate the role of NP tissue in the process of spinal fusion *in vivo*.

Materials and methods

Study design

Normal Danish Landrace pigs were chosen for the investigation, due to their size and availability. Ten female pigs, each 3 months old and weighing around 50 kg, were included in our study. Each pig underwent a two-level anterior lumbar interbody fusion of L3/4 and L5/6 with the employment of Brantigan I/F cages (8 mm; AcroMed, Cleveland, Ohio, USA). Each lumbar level was randomly selected for one of the following fusion methods: (1) implantation of Brantigan cage filled with autogenous iliac crest bone graft, or (2) implantation of Brantigan cage filled with a mixture of autograft and the NP tissue harvested from the disc level at which the cage was to be inserted. Each cage was secured with two staples (22*16; Howmedica GmbH, Schönkirchen, Germany). The pigs were observed for 3 months before sacrifice. The research protocol was approved by the Danish Board for Animal Research.

Anaesthesia

The animals were premedicated intramuscularly with 25 mg midazolam (Dormicum; Hoffman-La Roche, Basel, Switzerland) and 200 mg azaperon (Stresnil; Janssen Pharmaceutica, Beerse, Belgium). Orotracheal intubation was set up after intravenous injection of 20 mg etomidate (Hypnomidate; Janssen Pharmaceutica). Anaesthesia was sustained by the inhalation of isoflurane (1.5%).

Surgery

Under aseptic conditions, autogenous bone graft was taken from the left iliac crest with the pig placed in a right-sided recumbent position. The pig was then placed in a supine position with its legs tied to the table. The abdomen was prepared and draped, and a paramedian abdominal incision was chosen. The rectus abdominis muscle and its sheath were incised and retracted. The innermost layer, the fascia of transversus abdominis, was dissected with great care to avoid damage to the peritoneum lying immediately underneath.

After separation and retraction of the peritoneum and its contents, the quadratus lumborum and psoas major muscles were in view. The anterior lumbar spine was easily identified owing to its thick, shiny anterior longitudinal ligament. After ligation and the

cutting of the segmental vessels, the L3/4 and L5/6 intervertebral discs were excised together with the cranial and caudal endplates as well as part of the anterior longitudinal ligament by use of a special guide instrument designed by the present investigators. Nucleus pulposus was carefully collected from the level at which the mixture was to be grafted and then weighed. Bone graft was morselized and packed into the two cages in equal amounts. A cage was randomly chosen, and the bone graft was pushed out again and mixed thoroughly with the jelly nucleus pulposus to form a pellet. The pellet was again packed into the cage. Due to the compression applied, some of the jelly NP tended to be squeezed out from the graft surfaces and the side holes of the cage upon repacking. However, the cages were inserted without cleaning away the NP on the surface. Each cage was then secured with two staples. The abdominal muscle and the rectus abdominis sheath were carefully sutured, and the skin was closed by running sutures. Prophylactic ampicillin (1.0 g, Anhypen; Gist-Brocades, Delft, the Netherlands) was given before and immediately after surgery (1.0 g, I.V.) and analgesic buprenorphine (Temgesic; Hull, UK, 0.3 mg, I.M.) was given post-operatively twice a day for 3 days.

Triple fluorochrome labelling and sacrifice

Triple fluorochrome labelling with alizarin (25 mg/kg), tetracycline (20 mg/kg) and calcein (20 mg/kg) was given 3 days, 2 weeks and 6 weeks before sacrifice. Pigs were sacrificed after 12 weeks under general anaesthesia by I.V. injection of pentobarbital (0.4 mg/kg).

Radiographic examination

Plain radiographs of anterior-posterior and lateral views were taken immediately after operation and at termination 3 months later. After sacrifice, the whole lumbar spinal column, from L1 to L7 was removed en bloc, stripped of soft tissue, and transported to the laboratory and stored under -20°C until examination.

Computed tomographic evaluation

Two-millimetre-slice sagittal and cross-sectional computed tomographic (CT) scans were made on each lumbar spine specimen. CT images were evaluated by two independent observers. Fusion was defined as a continuous bone bridge across the cage with no obvious disruption, as signified by a radiolucent line in at least one of the serially obtained sagittal images.

Histomorphometry

The cages were harvested together with neighbouring vertebral bone. Then they were split into left and right halves sagittally. One part was randomly chosen for histomorphometric processing, and the other was used for fluorochrome study. Specimens for histomorphometric study were dehydrated in graded ethanol (70–99%) containing 0.4% basic fuchsin, and then embedded in PMMA. Sections were cut with 500 μm in between, to obtain the maximal range of sampling. Each section was cut to a thickness of 50 μm using the Sawing Microtome KDG 95 (Meprotech, Heerhugowaard, the Netherlands). The surface was counterstained with 2% light green for 2 min. Four slides were produced from each specimen for histomorphometric study. Blinded quantitative evaluations of slides were performed using the linear intercept technique (CAST-Grid software, Olympus Denmark A/S, Glostrup, Denmark). Bone volume, bone marrow space and fibrous tissue were calculated in percentages.

Statistics

Data were analysed by SPSS and are presented as mean values $\pm$ standard deviation (SD). The Q-Q plot (a normality test) for an approximation to normal distribution was utilized. The paired *t*-test was generally applied for normally distributed data sets, to compare different levels. Fusion rates were compared with Fisher's Exact Test; $P < 0.05$ (two-tailed) was considered significant.

Results

Surgery

In this study of ten pigs, no intraoperative nor postoperative complications were observed. The operations lasted between 170 and 270 min (mean 223 min). Anaesthesia by the inhalation of isoflurane (1.5%) established a fairly stable condition during the entire operation. The average amount of NP obtained to mix into the autograft was 214 mg (SD 78 mg). The amount of autogenous bone graft was 1.09 g (SD 0.07 g) in pure autograft levels and 1.09 g (SD 0.08 g) in the autograft/NP levels. The surgery itself, including the bone graft harvesting and anterior interbody fusion, was well tolerated by the pigs. Most of the pigs could stand up and were mobile the following day with the help of analgesics. The total body weight of the pigs increased from 45.4 kg (SD 5.4 kg) at the start to 55.1 kg (SD 5.6 kg) at sacrifice.

Radiograph

Radiographs of the specimens revealed no obvious kyphosis or any implant migration. A thin bony bar across the cage could be observed in some instances (Fig. 1) Bone formation was seen around some of the cages and staples. Though the carbon-fibre cage is radio-transparent, judgement of the osseous union inside the cages remained difficult.

CT scanning

CT was found useful in determining the fusion status inside the cage. The carbon-fibre cages produced no artifacts. Sagittal images clearly demonstrated the bone inside the cages (Fig. 2). The levels with pure autograft had a higher rate of fusion (7/10) compared to those with autograft/NP (2/10), but the difference was not significant ($P = 0.07$, Fisher's Exact Test, two-sided).

Histomorphometry

Histologic fusion with continuous trabecular bridging across the cage was observed for 70% (7/10) of the pure autograft levels. However, the fusion rate for the autograft/NP lev-

Fig. 1A,B Plain radiograph from pig no. 9. **A** Lateral image immediately after operation demonstrates the cage-staple construct. **B** Three months later, the cages and staples are still in position and a bony bar can be seen inside the cage on the lower level (L5/6, pure autograft level)

Fig. 2 Sagittal computed tomographic (CT) image of the lumbar spine of pig no. 6, 3 months after operation. The pure autograft level (L3/4, upper level) had a better bone ingrowth than that of the autograft/nucleus pulposus (NP) mixture level (L5/6, lower level)

els was only 10% (1/10). At one of the lumbar levels with autograft/NP, a thin line of fibrous tissue in the middle of the cage could still be observed, which was not discerned on CT image. The difference was significant ($P = 0.02$,

Fig.3 Comparison of histomorphometric results inside the cages from the two implantation methods. The results show significant differences in new bone formation ($P=0.04$), bone marrow space ($P=0.024$), and fibrous tissue ($P=0.048$, paired t -test)

Fisher's Exact Test, two-sided). The pure autograft levels appeared to be more mature in their trabecular bone quality and had a higher percentage of new bone formation and bone marrow space, while having a lower percentage of fibrous tissue (Fig. 3). New bone formation was found to start at neighbouring vertebral bodies and extend toward the centre of the cages. The trabecular bone was oriented longitudinally as a result of its bearing weight in the well-fused cages (Fig. 4). Graft remnants were not present in any of the cages after 3 months.

Calcein green could be seen throughout the cages, which revealed that new bone apposition had already taken place even at the centre of the cage at the time of injection (6th week). Alizarin red line was faintly seen as the third line. Quantitative analysis was precluded by the very thin or thick lines and irregularity of the labelling lines present.

Discussion

The pig model has often been used for laparoscopic spine surgery training and experimental studies [11, 12]. In the present study the pig model was employed because of the availability and size suitability of the pigs for the cages. Human surgical techniques were used with no modification of the standard instruments. The only disadvantage was that the pig is a growing animal. In our experiment, however, we carefully excised the cranial and caudal endplate of the fusion level with the guide of a self-designed instrument, and fed the pigs with controlled diet to avoid rapid growth of the vertebra. The mean total body weight increased only 21.3% after 3 months.

The study design here entailed mixing the bone graft and the NP tissue inside the cage. We understand that in

Fig.4A,B Histologic section micrographs from pig no. 5, 3 months postoperatively. The cage with the mixture of autograft and NP (A) did not fuse, while the cage with autograft only (B) showed complete fusion ($\times 1.25$ magnification)

clinical situations, NP tissue is never intentionally mixed with the bone graft in such a large quantity inside the cage. We chose to set up this extreme experiment condition to elucidate whether the NP tissue can affect the fusion in a well-controlled environment, in which the amount of NP tissue can be measured and the results can be reproduced.

Our plain radiographs demonstrate no severe deformity of the spine and no cage displacement. However, in a pilot study, in which no supplementary fixation was used, we found that one of the cages displaced anteriorly to the spine column, and in two of the pigs, absolutely no bone was formed inside the cages after 6 weeks. Hence, two staples were employed for supplemental fixation of each cage. Providing a stable mechanical environment has been shown in minipig and canine models to lead to a histologically and biomechanically superior healing and more rigid spinal segment [3, 16]. Transpedicular instrumentation has also been used in an animal interbody fusion study in sheep [10]. However, transpedicular instrumentation was not practical for our two-level anterior interbody fusions due to the extended operation time and greater blood

loss involved. The two staples used were easy to apply and did not take much space anteriorly, thus avoiding irritating the abdominal aorta. We did not find any cage displacement or migration in these ten pigs.

The Brantigan cages offer both an ideally confined environment and the property of radio-transparency. Newly formed bone in the cage could be seen on plain radiographs and CT scans. The CT results here showed that the pure autograft levels had a higher radiological union rate, but the difference was not significant. We consider that the reason is likely due to the limited number of animals and/or short observation time. The CT and histological evaluation results of fusion agree well, except in one level, where a thin line of fibrous tissue present in the middle of the cages was hard to discern on the CT image. For evaluation of the bone inside the cage, however, plain radiographs could only give a gross image, which made quantification very difficult. Even though our specimens have the advantage of being a clear spine column without any abdominal organs or muscles to give shadows, access is easier with sagittal CT scanning, as bone formation can be evaluated continuously and union versus non-union is well defined.

The histomorphometric results showed significant differences between the levels of pure autograft and those with autograft/NP. Bone formation was significantly decreased or delayed by mixing the NP into the autograft. The underlying mechanism is not clear. Is it simply caused by the fibrous tissue formation that can be seen when other tissue, such as fat or muscle, is mixed into the autograft? We packed the cages of both the pure autograft and NP/autograft levels with the same amount of bone. The rest of the space inside the cages would be filled either by the jelly NP, in the NP/autograft cages, or with blood clot in the pure autograft cages. Following the process of vascular invasion, both NP jelly and blood clot would be substituted by fibrous tissue. It is hard to ascribe the reason to simple tissue barrier. Furthermore, tissues like fat or muscle can hardly be compressed, and would definitely take up some space inside the cage, which would make repacking of the same amount of bone graft impossible if we were to use them as controls. Being avascular, the nucleus pulposus is normally exempt from the effects of the immune system. Leakage of the NP through herniation or surgery can result in a local inflammatory reaction and ac-

tivation of immunocompetent cells [6]. When cultured in vitro, NP can also secrete cytokines such as IL-1 β , IL-6 and IL-10 [14]. Our previous in vitro study showed that frozen NP tissue can stimulate the metabolism of osteoblast-like cells, and we also found the presence of IL-6 in the culture medium [9]. These cytokines could have complex effects on osteoblast metabolism [17]. The exact underlying mechanism could not be drawn from the present study. The difference between our in vivo effect and the in vitro effect can possibly be explained by the absence of any immune system in vitro. We tried to show the inflammatory response by histoimmunochemical method on our slides, but due to our thick sections and the embedding method, we did not succeed in the staining.

Biomechanical testing was not performed, because we wanted to keep an intact interface between the host bone and the bone graft. Furthermore, callus formation around the cages produces great variations in the mechanical results. Our main purpose was to test whether the NP tissue could influence the bone graft healing. That was also the reason why we chose the staining method described by Gotfredsen et al. [5], which enabled us to differentiate between newly formed bone and soft tissue histologically. The results showed sufficient contrast.

The limitation of the present study is that we lacked the information that would be provided by a longer observation period. We terminated the pigs after 12 weeks, because we assumed at the beginning that the immunogenic and inflammatory characteristics of NP would mean it should have an early effect. The early results do show a significant difference; however, we do not know whether this difference is transient or not. Diedrich et al. [2] reported, in a clinical follow-up on posterior lumbar interbody fusion with cages, that the fusion rate tends to increase over time, even after 2 years. We are not able to tell whether our unfused cages would finally have fused.

We conclude from this experiment that bone formation can be decreased or delayed inside the Brantigan cage if NP is mixed into the bone graft in large quantities in the pig model.

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