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

Haisheng Li
Xuenong Zou
Malene Laursen
Niels Egund
Martin Lind
Cody Bünger

Received: 17 October 2001
Revised: 11 March 2002
Accepted: 24 May 2002
Published online: 29 August 2002
© Springer-Verlag 2002

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 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-Broca, 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.

#### 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 para-median 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-Broca, 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 50 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.