Original article

Anterior cervical discectomy and interbody fusion with a dentate titanium cage: An experimental radiological and histopathological study in pigs

Tsitsopoulos PD[1], Tsonidis ChA[1], Tsitsopoulos PPh[1], Mintelis AI[1], Psalla DA[2], Desiris AK[3]

[1]2nd Department of Neurosurgery, Hippokratio General Hospital, Aristotle University School of Medicine, Thessaloniki, Greece, [2]Department of Pathology, Aristotle University, Veterinary School of Medicine, Thessaloniki, Greece, [3]Department of Surgery, Aristotle University, Veterinary School of Medicine, Thessaloniki, Greece

Abstract

Background - Aim: The distraction and stabilization provided by anterior cervical discectomy and fusion contribute to neural decompression and optimize osteogenesis. A new titanium cervical implant with specific properties was applied through an anterior approach in ten pigs. Implant behavior regarding in situ position and related osteogenesis were evaluated. Methods: In this controlled animal study, the progress of fusion and osteogenesis was evaluated after one level cervical interbody fusion with a new titanium cage. Ten pigs underwent anterior cervical discectomy and fusion. No substitutes stimulating osteoblastic activity were used. Plain radiographs were carried out. The animals were euthanised 12 and 14 weeks after the operation respectively. Tissue samples were processed routinely and studied histologically. Results: All pigs survived the surgery. Plain radiographs confirmed implant position. Histological analysis demonstrated fibrous connective tissue formation inside and around the implant which was largely transformed into cartilaginous and osseous tissue. Conclusions: Intervertebral space stabilization remains a parameter of crucial importance for early bone healing after anterior cervical discectomy and fusion. The new titanium alloy cage tested in this experimental study can offer the necessary stabilization for osteogenesis and adequate cervical interbody fusion without the need of growth factors. *Hippokratia 2006; 10 (4): 153-162*

Key words: *cervical spine, anterior cervical discectomy, fusion, titanium cage, grafts, osteogenesis, pig model*

Corresponding author: *Tsitsopoulos PPh, Dpt of Neurosurgery, Hippokratio General Hospital, Konstantinoupoleos 49, P.C. 54642, Thessaloniki, Greece, e-mail: par_tsits@yahoo.gr, Tel: +30 2310892332, Fax: +30 2310992945*

Introduction

Anterior cervical surgery first gained popularity in the mid-1950's as a method of treatment for cervical disc disease[1,2]. Since that time, the indications have expanded to include degenerative conditions, treatment of cervical kyphosis, decompression and stabilization of cervical trauma and resection of cervical neoplasms[3]. The need for fusion in a single cervical level remains controversial. Some authors suggest that if the operation is carried out for a laterally herniated "soft" disc, especially in a young patient, no fusion is required[4]. On the other hand, fusion maintains disc space height, prevents disc space collapse and stabilizes the intervertebral space while encouraging osteogenesis[5].

Titanium alloy cages have been widely used for spinal reconstruction since 1986 when they were first introduced. They serve as structural devices containing autologous local bone or iliac crest bone graft, obviating the need to harvest large structural bone grafts[6]. Recently, non-autologous bone graft in combination with other bone growth factors have been additionally used[7]. Although success rates in anterior cervical surgery are generally high, problems such as graft extrusion or collapse, bone graft donor side morbidity and pseudarthrosis continue to challenge[3]. The main disadvantage of titanium implants is the difficulty in assessing fusion status. Additionally, the potential risk of damaging surrounding soft tissue structures in case of cage extraction, created the need to secure the cage in place with cervical plate and screws even after a single-level cervical surgery[8]. In this way, surgical instrumentation turned to be complicated and time consuming, potentially increasing the risk of infections.

The purpose of this paper is to describe the progress of osteogenesis noticed on a very well achieved stabilization after a single-level anterior cervical surgery with fusion using a new intervertebral titanium cage. The stabilization obtained with this cage creates optimum conditions for adequate bone regeneration and fusion. Based on this hypothesis, anterior cervical discectomy and fusion in ten pigs was performed. The osteoblastic activity was evaluated at two different points in order to assess the progression of bony fusion.

Material - Methods

Study design. Using the pig model a newly designed titanium intervertebral implant was applied in the cervical spine of ten 5 month-old Danish Landrace female pigs. The average weight of the pigs was 68.2 kg (range 62.3-74.7 kg, SD: 2.6). The procedure was performed at C_5-C_6 level. Multiple levels study on the same spine was excluded because of the potential risk of producing subsequent spinal instability. The research protocol was approved by the local authorities.

Cage information: PARM implant (named after the inventor's son) is a cylinder with an outer periphery less than the periphery of the vertebral body so that the prosthesis interposes in between the cervical vertebral bodies after removal of the intervertebral disc. It is engineered for optimal anatomical fit to the vertebral endplates.

The ring wall thickness of the prosthesis is 2 mm, the external ring diameter 13 mm and the internal ring diameter 9 mm. Four available models exist. In the present study the anteroposterior implant height was 5.5 mm to 7.0 mm including dents. Dent height is 1.49 mm. The central opening of the prosthesis leaves a satisfactory space for bone formation inside the ring. In order to further facilitate osteoconduction and bone growth the available cages have holes on their later side (Figure 1).

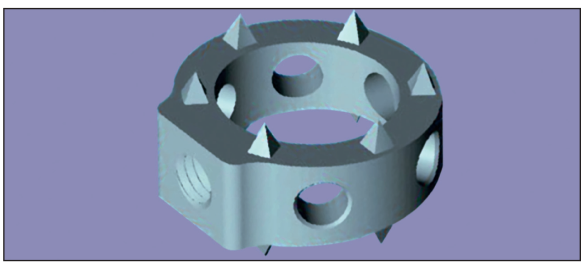

Figure 1. *The PARM cervical titanium cage*

All cages are made of TI6AL4V (ISO 5832-3 Implants for surgery - Metallic materials - Part 3: Wrought Titanium 6 - Aluminium 4 - Vanadium alloy).

Anesthesia: Xylazine was administered intramuscularly (1mg/kgr of body weight, IM). Fifteen minutes (15æ) later, ketamine HCl was given IM (15 mg/kg of body weight). Anesthesia was induced via intravenous administration of Sodium Pentothal (15 mg/kg of body weight, IV). Tracheal intubation followed and anesthesia was maintained with a mixture of Halothane in Oxygen and Fentanyl Citrate IV.

Surgical technique: The animals were kept in a supine position; the operating field was prepared from the submandibular area to the base of the neck. Under aseptic conditions, a longitudinal left anterolateral skin incision was performed, the subcutaneous fascia was incised and dissection of the area muscles followed. Trachea, esophagus and common carotid artery were retracted laterally and the desired intervertebral space was identified. Hemostasis was achieved with bipolar cauterization. A standard right anterior approach procedure was performed to the appropriate disc space. After incising the fibrous ring of the intervertebral disc, discectomy was carried out posteriorly to the level of the posterior longitudinal ligament with total removal of the nucleus pulposus and most of the fibrous ring. The intervertebral space was spread apart with Caspar screws device, placed on the anterior and posterior vertebrae. Freshening of the anterior and posterior endplates followed with the use of curette until exposure of cancellous bone. The titanium cage was inserted and properly positioned in between the vertebrae to fill the gap after disc excision. To test the stability of the cage, distraction was momentarily relaxed and the proper size of the implant was used according to the cervical level. The Caspar retractor was removed and the surgical wound was closed in layers.

Postoperatively all animals fully recovered from general anesthesia. The pigs were housed individually and fed with a controlled diet. All pigs were mobile the following day and no postoperative complications were observed. Ampicillin 1g/day IM was given pre- and postoperatively for the next 3 days. Nalbuphine Hydrocloride (15 mg IM) was given twice a day for 3 days as analgesic. The pigs were killed under general anesthesia via IV injection of potassium chloride overdose at the two time points postoperatively: five at 12 weeks and the rest five at 14 weeks after implantation. Anterior-posterior and lateral plain radiographs were taken immediately after the operation (Figure 2), at 4 weeks and at termination, at 12 and 14 weeks respectively (Figure 3).

Figure 2. *Plain radiograph. Lateral view immediately after operation. No obvious kyphosis or implant displacement is noticed.*

Figure 3. *Plain radiograph at termination time, 14 weeks after operation. Bone formation is visible around the cage with no obvious interruption by a radiolucent line. A thin bony bar into the cage is also observed through the peripheral openings.*

Part of the cervical spine was excised at the level of fusion. Tissue parts were obtained from inside and around the cage. The samples were collected, isolated and fixed in 10% phosphate-buffered formalin for 48 hours. Subsequently, they were further decalcified with the formic acid sodium-citrate method. Blocking in paraffin wax followed, 5μm sections were taken, stained with hematoxyline-eosine and studied under light microscope.

Results

Postoperatively the total body weight of the pigs increased from 68.2±2.6 kg) at operation time to 85.6±4.6 kg) at euthanasia time, that was a constant rate. Implant efficacy was evaluated radiographically and histopathologically.

Radiographic examination: Plain radiographs revealed no obvious kyphosis, displacement or implant migration and the normal lordotic angle of the cervical spine was maintained. Evaluation of bone formation inside the cage was impossible because the tested implant was not radiolucent. However, in all cases, a continuous bone bridge around the cage with no obvious interruption by a radiolucent line was created at 14 weeks, while a thin bony bar into the cage was visible through the peripheral cage openings in some instances (Figure 3). Bone formation was detected around all cages. Plain radiographs could give sufficient information only concerning gross structures, thus quantification of osteogenesis was impossible.

Histological study: Histopathological examination revealed development of tissue inside and around the implant (Figure 4). Specifically, 12 weeks after implantation predominantly cartilaginous and less osseous tissue were observed (Figure 5). Fourteen (14) weeks after implantation most of the newly formed connective tissue has been transformed to osseous tissue (Figure 6). Osteogenesis was similar in the area around the implant compared to the area inside the implant.

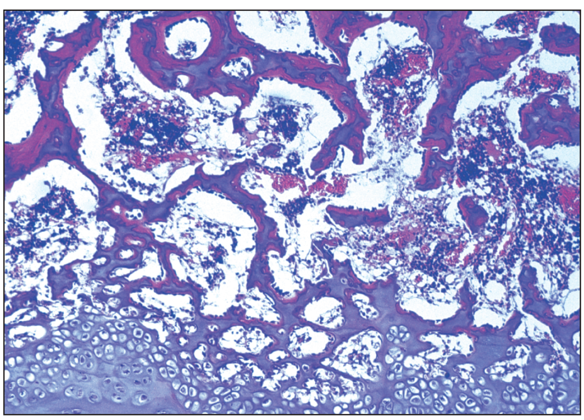

Figure 4. *Gross examination of the titanium cage 3 months after implantation revealed development of tissue inside and around the implant.*

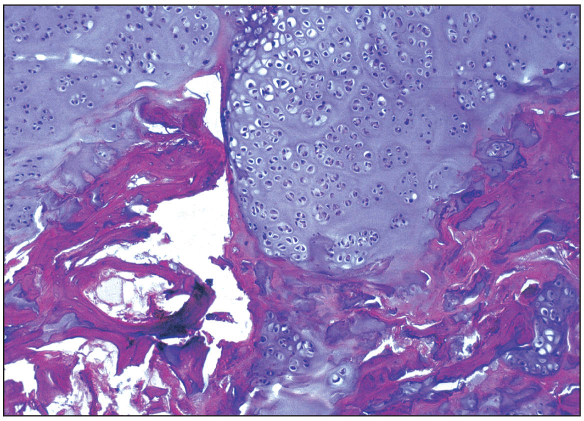

Figure 5. *Histological examination 12 weeks after implantation. Cartilaginous and osseous tissue have been formed (Hematoxylin-Eosine*200).*

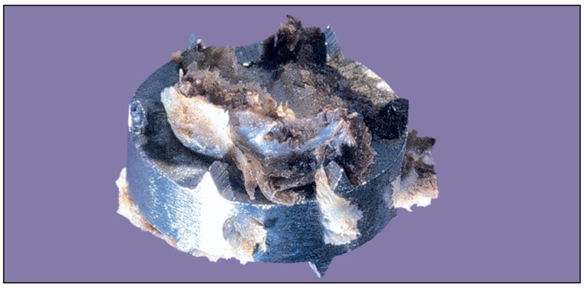

Figure 6. *Histological examination 14 weeks after implantation. Most of the connective tissue has been transformed to osseous tissue (Hematoxylin-Eosine*200).*

Discussion

In spine research the pig has been used as a model[9,13]. In the present study the pig was employed because of the availability and size suitability for the implants. Human surgical techniques were used with no modification of the standard instruments[14,15]. In this experiment, the pigs were nearly at the end of the growing period. Cranial and caudal endplates at fusion levels were carefully excised. Moreover, all pigs were fed with controlled diet to avoid rapid growth of the vertebra. The mean total body weight increased only 25.5%.

Recent protocols and research indicate that the faster the osteogenetic procedure starts, the better fusion is achieved[16-18]. For this reason a great variety of bone grafting options with or without application of fusion devices has been widely used. Autogenous bone grafts allogeneic bone grafts, xenograft bone, ceramic bone graft substitutes and hydroxyapatite blocks have been repeatedly evaluated for their usage in cervical spine surgery, while tantalum blocks are currently in clinical trials[3,4,7].

A great variety of fusion devices have become available and offer potential alternatives. Cages are usually made of biocompatible materials and provide good structural strength. Titanium mesh cages, cylindrical threaded cages, carbon fiber cages, with or without the addition of an anterior cervical plate have been also widely used[3,7,19-22,28]. Osteogenic growth factors like bone morphogenetic proteins, beta-tricalcium phosphate have now become an important research direction, minimizing the use of autografts and maximizing bone ingrowth[23-27]. A combination of techniques, including the use of interbody cages to achieve immediate stability and maintain intervertebral height and certain porous formulations of hydroxyapatite with recombinant bone morphogenic proteins to promote rapid fusion, might provide the best option[3]. On the other hand, the cost of such technology may be prohibitive, whereas possible health economic implications cannot be ignored[28].

The present newly designed titanium cage seems to provide fast and safe stabilization of the cervical spine, sufficiently supporting physiologic loads present on the neck, meeting all the mechanical requirements for prevention of collapsus and promotion of fusion. No other supplementary fixation seems to be required. Secure anchoring and no migration or any other displacement of the implant was noticed. It should be noted that neither the extreme mobility of pig's cervical spine nor its continuing activities provoked migration or displacement of the implant.

In minipig and canine model studies a stable mechanical environment has been shown to lead to a histologically and biomechanically superior healing and more rigid spinal segment[29, 30]. In this experimental study, early osteogenesis was noticed on plain radiographs with newly formed bone apparently demonstrated within as well as around the implant. No bony graft material or growth factor was placed inside the implant or within the interspace around it. Following the process of vascular invasion, blood clot is substituted by fibrous tissue as a result of a local inflammatory reaction and activation of osteoblastic cells, promoting osteogenesis and osseointegration procedures[31].

Several growth factors are considered to promote mitosis, angiogenesis and chemo taxis. In a relevant study, during a 3 month period time a reasonable fusion rate was observed with the application of BMP-2 or combined IGF-I/TGF-β1[32]. In our experiment, in the same time period, comparable results were noticed without use of any growth factor.

In human, the variety of clinical situations and the limitations imposed as age, sex, osteoporosis, menopause, smoking may compromise a positive result after anterior cervical discectomy and fusion. Prompt surgical technique for cage placement, minimal instrumentation, minimal allograft implantation and surgical time accommodation may promote a good, fast, and safe fusion and possibly have beneficial effect on patients outcome.

In conclusion the presented data indicate that a very important parameter for early enhanced bone healing after anterior cervical discectomy and fusion remains stabilization of the interbody space. The newly designed titanium cage tested in this animal study may offer by itself in human the necessary stabilization for early osteogenesis and adequate cervical interbody fusion.

Acknowledgements

The authors are indebted to Mr. Kalaitzis Ch (HELMED Spine Implants) for his valuable assistance in accomplishing the experiment.

References

1. Cloward R. The anterior approach of ruptured cervical discs. J Neurosurg 1958; 15:602-616
2. Smith G, Robinson R, Maryland B. The treatment of certain cervical spine disorders by anterior removal of the intervertebral disc. J Bone Joint Surg 1958; 40A:3, 607-624
3. Anderson G, Albert T. Bone grafting, implants and plating options for anterior cervical fusions. Orthop Clin N Am 2002; 33:317-328
4. Wilson DH, Campbell DD. Anterior cervical discectomy without bone graft. J Neurosurg 1997; 47:551-555
5. Greenberg M. Handbook of neurosurgery 5th edition Thieme medical publishers Inc, 2001, Stuttgard New York
6. Riew D, Rhee J. The use of titanium mesh cages in the cervical spine. Clin Orthop and Related Research 2002; 1:47-54
7. Wood E G III, Hanley E N Jr. Types of anterior cervical grafts. Orthop. Clin. N Am 1992; 23:475-486
8. Limbeek J, Jacobs W, Anderson P, Pavlov P. A systematic literature review to identify the best method for a single level anterior cervical interbody fusion. Eur Spine J 2000; 9:44, 129-136
9. Langmayr JJ, Seykora P, Jakober R, Klima G, Ortlier M. The interface between ionomer cement and bone in the porcine cervical spine. Acta Neurochir (Wien) 1996; 138:154-61
10. Li H, Zou X, Laursen M, Egund N, Lind M, Bunger C. The influence of intervertebral disc tissue on anterior spinal interbody fusion. An experimental study on pigs. Eur Spine J 2002; 11:476-481
11. Muhlbauer M, Ferguson J, Losert U, Koos WT. Experimental laparoscopic and thoracoscopic discectomy and instrumented spinal fusion. A feasibility study using a porcine model. Minim Invasive Neurosurg 1998; 41:1-4
12. Olinger A, Pistorius G, Lindemann W, Vollmar B, Hildebrandt U, Menger MD. Effectiveness of a hands-ontraining course for laparoscopic spine surgery in a porcine model. Surg Endosc 1999; 13:118-122
13. Takahashi T, Tominaga T, Yoshimoto T, Koshu K, Yokobori AT Jr, Aizawa Y. Biomechanical evaluation of hydroxyapatite intercerebral graft and anterior plating in a porcine cadaveric model. Biomed Mater Eng 1997; 7:121-127
14. Bauer R, Kerschbaumer F, Poisel S. Atlas of spinal operations. Thieme medical publishers Inc., Stuttgart, New York, 1993
15. Louis R. Surgery of the spine. Surgical anatomy and operative approaches. Springer, Berlin, Heidelberg, New York, 1983
16. Boden SD. Bone repair and enhancement clinical trial design:spine applications. Clin Orthop 1998; 355(suppl) 336-346
17. Cornell CN. Osteoconductive materials and their role as substitutes for autogenous bone grafts. Bone grafting and bone substitutes 1999; 30:591-598
18. Togawa D, Bauer T, Lieberman I, Lowery G, Takikawa S. Histology of tissues within retrieved human titanium mesh cages. Spine 2003; 28:246-253

19. Kandziora F, Plfugmacher R, Schafer J, et al. Biomechanical comparison of cervical interbody fusion cages. Spine 2001; 26:1850-1857
20. Matge G. Cervical cage fusion with 5 different implants :250 cases. Acta Neurochirg (Wien) 2002; 144:539-550
21. Steffen T, Tsantrizos A, Fruth I, Aebi M. Cages. Designs and concepts. Eur Spine J 2000; 9 (Suppl. 1):S89-S94
22. Wigfield C, Nelson R. Nonautologous interbody fusion materials in cervical spine surgery. Spine 2001; 26:687-694
23. Garcia-Bolao I, Merino J, Martinez A, Grau A, Alegria E, Martinez-Caro D. Effect of hypercholesterolaemia on platelet growth factors. Eur J Clin Invest 1996; 26:929-935
24. Gunzburg R, Szpalski M. Use of a novel beta-tricalcium phosphate-based bone void filler as a graft extender in spinal fusion surgeries. Orthopedics 2002; 25(5 suppl):s591-s595
25. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent 2001; 10:225-228
26. Ohyama T, Kubo Y, Iwata H, Taki W. Beta-tricalcium phosphate as a substitute for autograft in interbody fusion cages in the canine lumbar spine. J Neurosurg 2002; 97(3 suppl):350-354
27. Zou X, Xue Q, Li H, Bünger M, Lind M, Bünger C. Effect of alendronate on bone ingrowth into porous tantalum and carbon fiber interbody devices: an experimental study in pigs. Acta Orthop Scand 2003; 74:596-603
28. Majd M, Vadhva M, Holt RT. Anterior cervical reconstruction using titanium cages with anterior plating. Spine 1999; 24:1604-1610
29. Fuller DA, Stevenson S, Emery SE. The effects of internal fixation on calcium carbonate. Ceramic anterior spinal fusion in dogs. Spine 1996; 21:2131-2136
30. Ylinen P, Kinnunen J, Laasonen EM, et al. Lumbar spine interbody fusion with reinforced hydroxyapatite implants. Arch Orthop Trauma Surg 1991; 110:250-256
31. Cunningham BW, Kanayama M, Haggerty CJ. An in-vitro biomechanical analysis on the stability and stress interbody fusion implants. J Bone Joint Surg Br 1999; 81 (suppl): 132-133
32. Kandziora F, Pflugmacher R, Scholz M, et al. Comparison of BMP-2 and combined IGF-I/TGF-β1 application in a sheep cervical spine fusion model. Eur Spine J 2002; 11:482-493