International Orthopaedics (SICOT) (2014) 38:1993–1999
DOI 10.1007/s00264-014-2377-z

ORIGINAL PAPER

In vivo tricalcium phosphate, bone morphogenetic protein and autologous bone marrow biomechanical enhancement in vertebral fractures in a porcine model

Elena Manrique · David Chaparro · Juan Luis Cebrián · Luis López-Durán

Received: 19 April 2014 / Accepted: 6 May 2014 / Published online: 7 June 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract
Purpose Minimally invasive techniques that introduce cement and bone substitutes inside the fractured vertebral body are a new treatment line with clinically proven efficacy. However, mechanical behaviours between different fillers throughout fracture evolution is yet to be clarified, as many substances are available for introduction into the vertebral body fracture.
Methods We comparatively studied biomechanical properties of tricalcium phosphate, tricalcium phosphate with bone morphogenetic protein (rhBMP-7) and autologous bone marrow aspirate with rhBMP-7 in vivo to determine what substance is optimal for repairing vertebral lesions in a porcine model. This biomechanical study was carried out with an Instron-type testing machine. Data registered were necessary strength to reach vertebral fracture [Newtons (N)], shortening (millimeters) of the vertebra, energy absorption until vertebral fracture (Joules) and vertebral unit stiffnesss.
Results For statistical study, we used the SPSS 16 package at a significance level of α=0.05. In the presentation of the results, mean, standard deviation of mean, median and interquartile range (IQR) were analysed. Mean and standard deviation (SD) of strength in newtons (N) for the vertebral fracture are 756 N (SD = 253) in group 1, 1,500 N (SD = 1598) in group 2 and 1,230 N (SD = 1,598) in group 3. Stiffnesss after fracture was 229 N (SD = 123) in group 1, 277 N (SD = 135) in group 2 and 404 N (SD = 325) in group 3.
Conclusions The association of tricalcium phosphate and BMP-7 generates major vertebral resistance to external energy, the cause of such fractures. In such fractures, minor shortening occurs as soon as the vertebral body is fractured. Autologous bone marrow and BMP-7 provides increased biomechanical behavior, and the vertebral body is thus significantly strengthened.

Keywords Porcine model · Vertebral fracture · Tricalcium phosphate · Bone morphogenetic protein · Autologous bone marrow · In vivo Lumbar retroperitoneal approach

E. Manrique · J. L. Cebrián · L. López-Durán
Department of Traumatology and Orthopaedic Surgery, Hospital Clínico San Carlos, C/Profesor Martin Lagos s/n, 28040 Madrid, Spain

D. Chaparro
Deparment of Internal Medicine/Emergency, Hospital Clínico San Carlos, C/Profesor Martin Lagos s/n, 28040 Madrid, Spain

E. Manrique (✉)
Servicio de Traumatología y Cirugía Ortopédica, Hospital Clínico San Carlos, C/Profesor Martín Lagos s/n, 28040 Madrid, Spain
e-mail: manrique.elena@gmail.com

Introduction

Vertebral fractures (VF) are extremely common in the elderly [1, 2]. The lesion is characterised by a loss of vertebral height, which occurs when the transmitted load exceeds bone strength. Its frequency is very high, and loss of vertebral height > 15 % is reported in women > 50 years at a prevalences of 26 % [3]. The appearance of an osteoporotic vertebral fracture incurs a five-times greater risk of developing new spinal fractures [4]. Standard treatment for such fractures, based on rest and decreased physical activity, inhibits the stimulation of bone formation, with bone loss that can reach 2 % per week [5]. Osteoporosis is a major health problem affecting 25 % of the population in developed countries; 33 % of the population is at risk for low bone mass. As old age is the main cause of decreased bone mass, the incidence of VF is directly related to the increase in life expectancy of our population. As 50 % of osteoporotic fractures affects the spine, we face a growing increase in the incidence of VF, which is a challenge for orthopaedic surgeons regarding diagnosis and management of these types of injuries and a major problem for

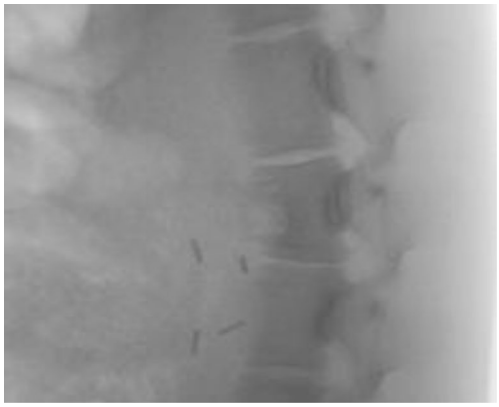

Fig. 1 Lateral view of vertebral defect

health authorities. This fact leads to increased resources for prevention, treatment and research and development of new techniques. The social and health consequences of VF are undoubtedly significant, not only for economic but for clinical implications for elderly patients: acute pain from moderate to severe intensity up to three months, decreased lung volume secondary to kyphosis, psychosocial consequences of disability, lack of adaptation to the environment, feelings of shame due to physical deformity, difficulty in performing daily activities, impaired quality of life (QoL), increased risk of spine fractures, increased rate of age-adjusted mortality up to 1.23-times higher in the case of one or more fractures and increased hospitalisation time considering that 25–40 % of patients diagnosed with VF are hospitalised at some point [6].

In our department, we are aware of this problem in daily clinical practice. Furthermore, it is anticipated that our healthcare resources will increasingly assume costs for this condition for years to come, trending upward with increase in life expectancy and osteoporosis as a true pandemic in developed societies on the one hand and on the other hand because actual management is far from optimally solving the problem of osteoporotic vertebral fracture.

In cases in which reduction of the VF is required, surgery is indicated. In addition to the risks involved with this type of surgery, the poor quality of osteosynthesis of osteoporotic bone does not allow the desired, optimal reduction. Open techniques have reduced indications in such patients and are limited to cases in which spinal instability or neurological deficit exists. Thus, treating osteoporotic fractures without surgical indication for VF remains palliative (bed rest, pain medication, physiotherapy) and immobilisation; conservative management for patients with osteoporotic VF has not been standardised [7]. The role of surgical treatment remains controversial and should be reserved for patients who fail initial nonsurgical management options. Patients with chronic pain > two months may be appropriate candidates for vertebral body augmentation, i.e. vertebroplasty (VP) or balloon-tamp reduction. Open surgical management with decompression and stabilisation should be reserved for the rare patient with neural compression and progressive deformity with neurologic deficit [8].

Supported by the results of using bone cement in cavities occupied by tumors [9], minimally invasive treatment for VF surgery are developing. These techniques have two theoretical advantages: adequate rigidity, and a decrease in the surgical aggression in elderly patients with comorbidity. The transpedicular VP involves percutaneous high-pressure injection of polymethylmethacrylate (PMMA) bone cement into the fracture through the pedicles of the fractured vertebra. Clinical results demonstrate pain relief, complication rates <10 % and increased vertebral body strength and stiffnesss [10], with complications secondary to cement extravasation. The lack of vertebral height recovery introduced by the surgical technique has prompted some changes in this procedure, such as including a bone impactor (bouncy ball) to recover vertebral body height and correct the deformity. This

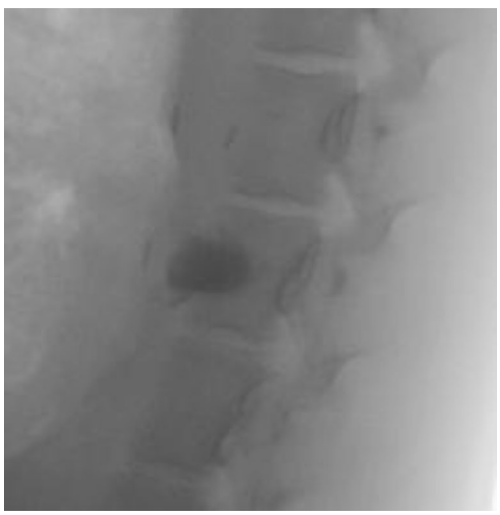

Fig. 2 Lateral view of vertebral defect filled with materials corresponding to each group immediately after surgery

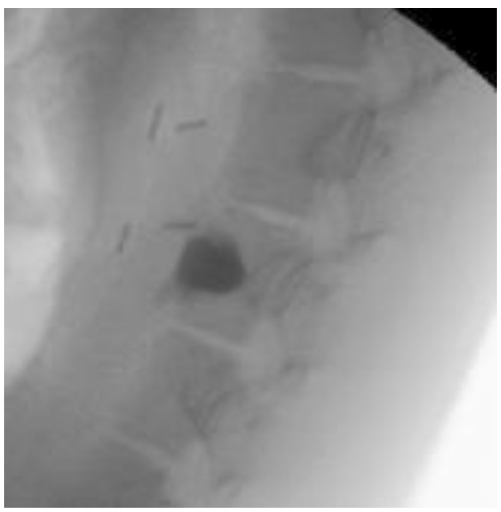

Fig. 3 Lateral view of the filled vertebral defect 30 days postsurgery

Table 1 Mean, standard deviation (SD) and minimum and maximum strength (Newton) values of each vertebrae until fracture

Strength	No.	Minimum	Maximum	Mean (SD)
Group 1	6	285	972	756 (253)
Group 2	6	110	4,322	1,500 (1,598)
Group 3	6	399	2,822	1,230 (1,054)

technique, known as balloon kyphoplasty (KP), allows the introduction of cement with low pressure, eliminating the complications of VP. The technique allows reduction with increased and maintained vertebral body height, a decrease in pain and improved patient activity level. However, due to its mechanical characteristics (rigidity) when used in osteoporotic spines, the introduction of intravertebral cement may cause fractures in adjacent vertebrae. VP and KP are effective procedures for reducing pain in osteoporotic vertebral fractures, and they have a similarly efficient effect on pain reduction. As correcting vertebral height and local kyphosis may have minimal effect on pain reduction [11], it is necessary to study other substances that, once introduced into the vertebral body, avoid these negative circumstances while allowing recovery of bone mass in the affected vertebra. Although evidence suggests that physical disability, general health and pain relief are better with VP and KP than attained with medical management within the first three months after intervention, high-quality randomised trials with two year follow-up are needed to confirm this. Furthermore, the reported incidence of symptomatic procedure-related morbidity for both VP and KP is very low [10, 12]. Other studies show that for long-term pain relief and functional improvement, there is no significant difference between KP and VP. Consistently, both interventions have similar risks for subsequent fracture and cement leakage. Thus, considering the higher cost of the KP procedure, VP could be more highly recommend over KP for treating osteoporotic VF [13].

Minimally invasive techniques that introduce cement and bone substitutes into the vertebral body are a new line of clinically proven treatment. However, mechanical behaviour of different fillers throughout fracture evolution remain to be clarified. Many substances may be introduced into the vertebral body fracture. We comparatively studied mechanical properties of tricalcium phosphate, tricalcium phosphate recombinant human bone morphogenetic protein (rhBMP-7) and autologous bone marrow aspirate with rhBMP-7 to determine which is optimal for repairing osteoporotic vertebral lesions [14–16].

Bone induction by osteogenic proteins (OP)/BMP is one of the most important discoveries in the field of bone physiology and bone surgery. OP and BMP are growth and differentiation factors capable of initiating recruitment, attachment, proliferation and differentiation of mesenchymal cells, leading to newly formed mature bone [17]. Clinical trials investigating long-bone applications have provided supportive evidence for the use of rhBMP-7 for treating open tibial fractures, distal tibial fractures, tibial nonunions, scaphoid nonunions and atrophic long-bone nonunions. Clinical studies investigating spinal fusion applications have provided supportive evidence for the use of this substance in posterolateral lumbar models and compromised patients as an adjunct or replacement for autograft. Both long-bone repair and spinal-fusion studies have demonstrated the efficacy and safety of rhBMP-7 [18].

Methods

Study design and surgical procedure

We used 18 female animals of the porcine species crossing Pietrain breed (this is a porcine model used for laboratory

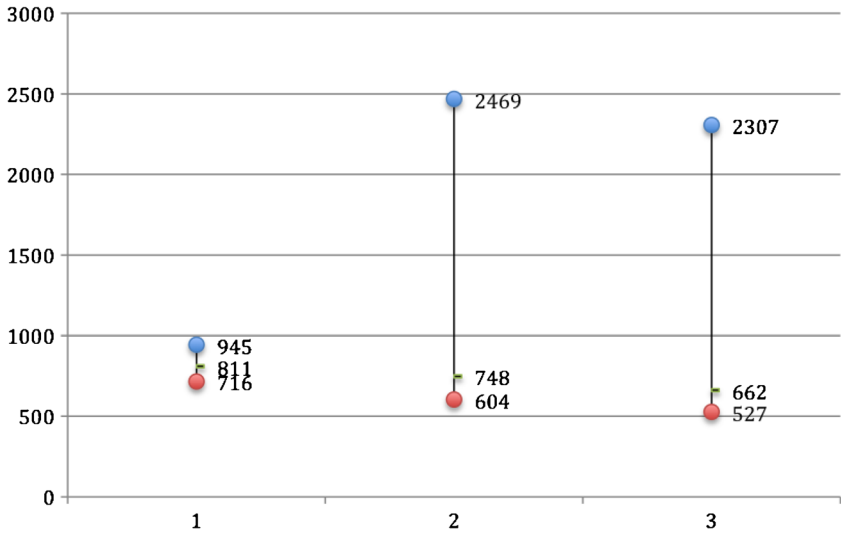

Fig. 4 Median and interquartile range (IQR) of strength required to fracture the vertebra

Table 2 Mean, standard deviation (SD), minimum and maximum in relation to vertebral shortening

Shortening	No.	Minimum	Maximum	Mean (SD)
Group 1	6	4.43	9.80	6.33 (2.06)
Group 2	6	2.81	11.75	6.72 (3.02)
Group 3	6	2.78	6.78	4.45 (1.55)

experiments). Throughout, the procedure followed the requirements of current legislation on protection of animals used for experimental purposes. Pre-operatively, pigs were randomly assigned to three groups—1, 2 and 3—consisting of six pigs each. We used general anesthesia with assisted ventilation. After the animal was anesthetised, it was placed in the right lateral decubitus position; a left paravertebral approach allowed retroperitoneal spine access. The peculiarity of the pig's lumbar spine consists of six vertebrae instead of the five in humans; however bone structure is very similar. The goal of this surgery was to reach the L3 vertebral body and create an anterior defect of 1 cm^3 (Fig. 1); this defect was subsequently filled (Fig. 2) with the material corresponding to group assignment: group 1, tricalcium phosphate; group 2, tricalcium phosphate and rhBMP-7; group 3, tricalcium phosphate and autologous bone marrow aspirate.

Thirty days after surgery, we recorded animal weight and complications, and the animal was euthanised. Then, radiograph were taken of the lumbar spine in anteroposterior and lateral projections (Fig. 3) vertebral unit consisting of the default vertebra (L3), and the upper and lower vertebrae (L2 and L4), free of muscular insertions, was removed immediately.

Biomechanical assessment

Immediately after removing the vertebral unit, a biomechanical study was carried out. The corresponding specimens, comprising proximal (L2) and distal (L4) vertebrae fixed in resin blocks with stainless steel screws for incorporation in the measuring machine, were prepared. We used an Instron type testing machine for small parts with axial loading (displacement 0.5 cm/min). Data were recorded digitally and graphically and created a table detailing the necessary strength [Newtons (N)], shortening (millimetres), absorbed energy (Joules) and vertebral unit stiffness (*see* "Results" section.

Results

Data analysis

For the statistical study, we used the SPSS 16 package and a significance level of α=0.05. Mean, standard deviation (SD) of the mean, median and interquartile range (IQR) were analysed. Analysis of variance (ANOVA) was not used because of the dispersion of the data within each group. Using the Wilcoxon formula, we determined statistical significance when comparing each variable between groups. Variables analysed were strength, shortening, stiffnesss and energy absorbed

– Strength:

Minimum strength (N) necessary to fracture the vertebra and group comparisons. In such small groups, it is important to evaluate the dispersion of elements. Table 1 shows SD and the element with maximum and minimum values in each group. Median and IQR for each case are shown in Fig. 4. There were no statistically significant differences between groups.

– Shortening:

Measuring resistance and strength, we assessed the decrease in vertebral height using the minimum strength

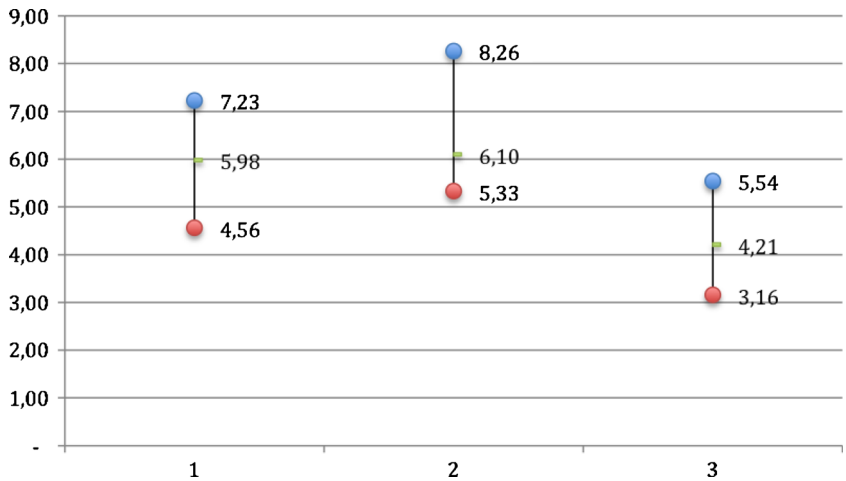

Fig. 5 Median and interquartile range (IQR) of vertebral shortening after breakage in each group (cm)

Table 3 Average, standard deviation (SD), minimum and maximum with regard to vertebral stiffnesss

Stiffnesss	No.	Minimum	Maximum	Mean (SD)
Group 1	6	49	361	229 (123)
Group 2	6	104	448	277 (135)
Group 3	6	89	936	404 (325)

necessary to cause breakage. These are important elements in recovery of a fractured vertebra. We determined mean values, and results are shown in Table 2. Variability and dispersion were measured using SD and IQR. Median and IQR also were analysed (Fig. 5).There was no significant difference between groups.

– Stiffnesss:

Stiffnesss refers to the aptitude of a structural element to support effort without displacement or deformation. It is another important variable after applying enough pressure to a vertebra to cause fracture. Mean, SD, minimum and maximum values are shown in Table 3. Mean and IQR are shown in Fig. 6. Wilcoxon's formula showed no statistically significant differences between groups.

– Absorbed energy:

Absorbed energy is the amount of energy absorbed by a vertebral unit when submitted to breaking pressure. SD and minimum and maximum values for each group are shown in (Table 4). Median and IQR are shown in Fig. 7. There was a statistically significant difference between groups 1 and 2 only.

Discussion

The use of tricalcium phosphate is an important advance in the management of osteoporotic vertebral fractures. This material has mechanical benefits similar to PMMA cement but with the added benefit of being a better osteoconductor, with the best bony apposition and final incorporation of bone, creating resistance strength similar to that of healthy bone, which resulted in tricalcium phosphate displacing cement for managing such fractures.

Our objective in this study was to determine the best element available for biomechanical bone reconstitution. We compared three groups in a porcine model by creating a vertebral-body defect , filling the defect is one group with tricalcium phosphate, in another with tricalcium phosphate plus rhBMP-7 and the third with tricalcium phosphate and autologous bone marrow aspirate.

Few trials report combining rhBMP-7 with tricalcium phosphate, and the ones that do exist assess its use for treating tibial nonunions [17, 19] or mandibular defects in rabbits [20]. Very good results are achieved with this combination, as it avoids the use of additional osteosinthesis systems. The novelty of this study is the application of this combination to vertebral defects, and preliminary results show that in this location, it can improve bony resistance. Clinical trials investigating long-bone applications provide supportive evidence for the use of rhBMP-7 for treating open tibial fractures, distal tibial fractures, tibial nonunions, scaphoid nonunions and atrophic long-bone nonunions. Clinical studies investigating spinal fusion applications provide supportive evidence for using rhBMP-7 in posterolateral lumbar models and compromised patients as an adjunct or replacement for autograft. Both long-bone repair and spinal-fusion studies demonstrate the efficacy and safety of rhBMP-7 [18], alone or in combination with autologous bone graft. The success of the rhBMP-7 autograft reached 83.3 % compared with 76.5 % for standalone rhBMP-7 in some series [21].

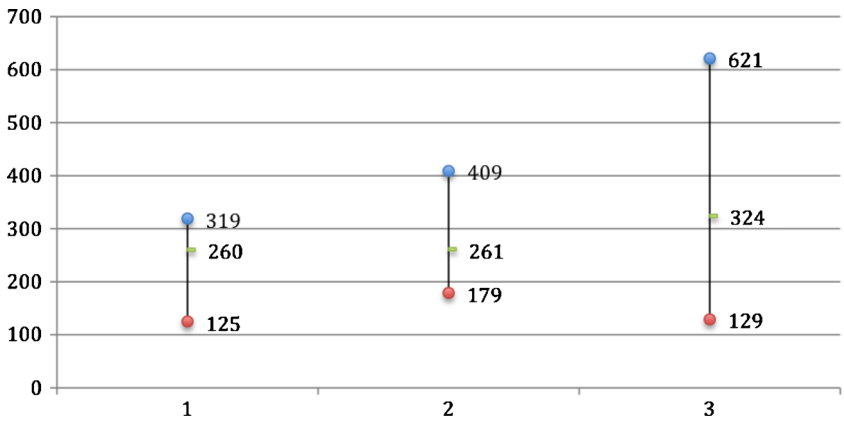

Fig. 6 Median and interquartile range (IQR) of vertebral stiffnesss (Newton/cm)

Table 4 Mean, standard deviation (SD), minimum and maximum for energy absorbed by the vertebra

Absorbed energy	No.	Minimum	Maximum	Mean (SD)
Group 1	6	2.31	8.87	6.15 (2.59)
Group 2	4	8.50	48.59	21.29 (18.53)
Group 3	5	2.92	29.03	10.84 (11.53)

The most important information obtained from our results is the number of study subjects. Three groups were created, and total of six specimens was analysed from each group. Each group contained a small number of subjects, which created a statistically important dispersion of information within each group. Due to the small number of subjects, we could not use ANOVA, so we used average and median analyses. Our analysis shows no statistically difference exists groups. However, results show enough differences between groups to indicate the possibility of different outcomes if more subjects were assessed.

To discuss results of the different variables, it is necessary to study two groups with similar variables to determine the line of argumentation. On the one hand, strength necessary to fracture the vertebra and energy absorbed by that vertebra indicate the minimum amount of energy necessary to surpass the limit of resistance of the damaged vertebra. On the other hand, shortening and stiffnesss indicate resistance of the damaged vertebra as soon as the limit of resistance is exceeded.

When the first two variables were evaluated, even though there was no statistically significant difference, good results were obtained after filling the defect with tricalcium phosphate plus rhBMP-7. This indicates that the resistance generated by the association of tricalcium phosphate and rhBMP-7 is bigger than that for the other studied elements. This resistance generates major support for the disabled vertebra and improves its consolidation. This element also generates resistance faster than the other elements studied. This information is not surprising if compared with the addition of tricalcium phosphate alone, but it is important when comparing the association of autologous bone marrow and rhBMP-7. Also interesting that when we analysed the other two variables, the association of autologous bone marrow and rhBMP-7 was better than the other two groups. This means that when bony resistance is exceeded and a fracture appears in the vertebral body, the bone with the better quality was the one filled with autologous bone marrow plus rhBMP-7, as shortening was minor and stiffnesss was major. Thus, this combination presents better biomechanical resistance against vertebral fracture. rhBMP-7 used alone in osteoporotic VF has the potential of inducing a rapid increase in bone strength locally at the fractured area [22]. Results can improve even further with the addition of autograft.

It is somewhat difficult to extrapolate these results to an osteoporotic vertebra, as it is necessary to look for major resistance to an external stimulus. Also, supposing there always exists a stimulus capable of causing a VF during simple activities of daily living, finding filling material with better biomechanical characteristics and maximum resistance is desirable. Future studies on individual animals with induced osteoporosis are necessary to identify significant differences between biomaterials used to fill osteoporotic VF.

This preliminary data suggest that a therapeutic effect on VF can be achieved with local administration of autologous bone marrow plus rhBMP-7, but further research is necessary to determine its effectiveness for improving bone mineral density and bony volume in an osteoporotic vertebral body.

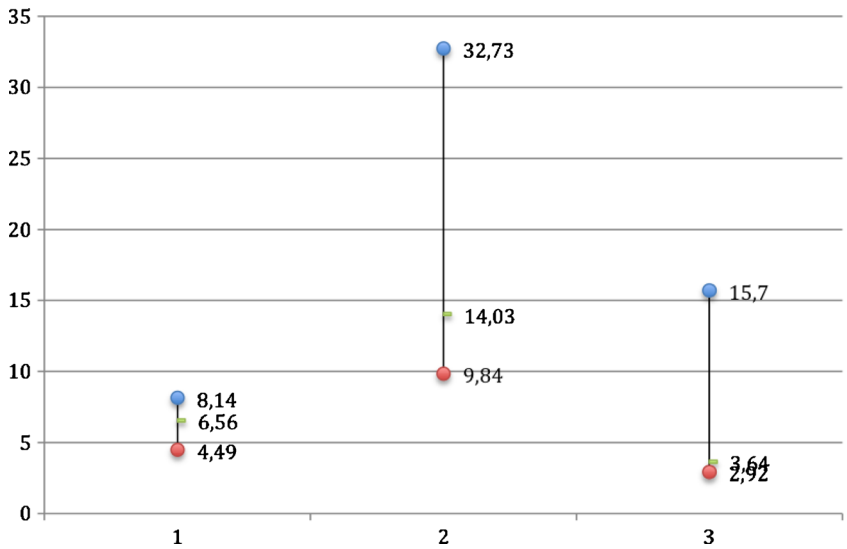

Fig. 7 Median and interquartile range (IQR) of absorbed energy

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Riggs BL, Melton LJ 3rd (1995) The worldwide problem of osteoporosis: insights afforded by epidemiology. Bone 17:S505–S511
2. Silverman SL (1992) The clinical consequences of vertebral compression fracture. Bone 13:S27–S31
3. Melton LJ 3rd, Kan SH, Frye MA et al (1989) Epidemiology of vertebral fractures in women. Am J Epidemiol 129(5):1000–1011
4. Cooper C, Atkinson EJ, O'Fallon WM, Melton LJ 3rd (1992) Incidence of clinically diagnosed vertebral fractures: a population based study in Rochester. J Bone Miner Res 7(2):221–227
5. Lindsay R, Silverman SL, Cooper C et al (2001) Risk of new vertebral fracture in the year following a fracture. JAMA 285(17): 127–132
6. Lyles KW, Gold DT, Shipp KM, Pieper CF, Martinez S, Mulhausen PL (1993) Association of osteoporotic compression fractures with impaired functional status. Am J Med 94:34–40
7. Longo UG, Loppini M, Denaro L, Maffulli N, Denaro V (2012) Osteoporotic vertebral fractures: current concepts of conservative care. Br Med Bull 102:171–189
8. Kim DH, Vaccaro AR (2006) Osteoporotic compression fractures of the spine; current options and considerations for treatment. Spine J 6(5):479–487
9. Galibert P, Deramond H, Rosat P, Le Gars D (1987) Preliminary noTe on the treatment of vertebral angioma by percutaneous acrylic vertebroplasty. Neurosurgery 33:166–168
10. Garfin S, Yuan HA, Reiley MA (2001) New technologies in spine: kyphoplasty and vertebroplasty for the treatment of painful osteoporotic compression fractures. Spine (Phila Pa 1976) 26:1511–1515
11. Dong R, Chen L, Tang T et al (2013) Pain reduction following vertebroplasty and kiphoplasty. Int Orthop 37(1):83–87
12. McGirt MJ, Parker SL, Wolinsky JP, Witham TF, Bydon A, Gokaslan Z (2009) Vertebroplasty and kyphoplasty for the treatment of vertebral compression fractures: an evidenced-based review of the literature. Spine J 9(6):501–508
13. Han S, Wan S, Ning L, Tong Y, Zhang J, Fan S (2011) Percutaneous vertebroplasty versus balloon kiphoplasty for treatment of osteoporotic vertebral compression fracture: a meta-analisis of randomised and non-randomised controlled trials. Int Orthop 35(9):1349–1358
14. Hillmeyer J, Meeder PJ, Nöldge G et al (2004) Balloon kyphoplasty of vertebral compression fractures with a new calcium phosphate cement. Orthopade 33(1):31–39
15. Lim TH, Brebach GT, Renner SM et al (2002) Biomechanical evaluation of an injectable calcium phosphate cement for vertebroplasty. Spine 27(12):1297–1302
16. Phillips F, Turner AS, Seim HB 3rd et al (2006) In vivo BMP-7 (OP-1) enhancement of osteoporotic vertebral bodies in an ovine model. Spine J 6(5):500–506
17. Pecina M, Giltaij LR, Vukicevic S (2001) Orthopaedic applications of osteogenic protein-1 (BMP-7). Int Orthop 25(4):203–208
18. White AP, Vaccaro AR, Hall JA et al (2007) Clinical applications of BMP-7/OP-1 in fractures, nonunions and spinal fusion. Int Orthop 31(6):735–741
19. Cubitt J, McAndrew A (2010) Management of tibial non-union with tricalcium phosphate and BMP 7. BMJ Case Rep. doi:10.1136/bcr.02.2010.2777
20. Busuttil K, Ayoub A, McMahonb J et al (2012) Mandibular reconstruction in the rabbit using beta-tricalcium phosphate (b-TCP) scaffolding and recombinant bone morphogenetic protein 7(rhBMP-7) e Histological, radiographic and mechanical evaluations. J Craniomaxillofac Surg 40(8):e461–e469
21. Giannoudis P, Dinopoulos H (2010) Autologous bone graft: when shall we add growth factors? Foot Ankle Clin 15(4):597–609
22. Kanakaris NK, Petsatodis G, Tagil M, Giannoudis PV (2009) Is there a role for bone morphogenetic proteins in osteoporotic fractures? Injury 40(Suppl 3):S21–S26