Effect of a single dose of pamidronate administered at the time of surgery in a rabbit posterolateral spinal fusion model

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Received: 17 September 2009 / Revised: 11 January 2010 / Accepted: 15 January 2010 / Published online: 3 February 2010
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Abstract Spinal fusion is usually performed on patients who receive bisphosphonates (BP); however, limited data on their action on spinal fusion are available. Previous studies in animal models have shown that chronic administrations of BP reduced spinal fusion rates, and only one study has shown that a single dose administration of zoledronic acid increased fusion rate. The objective of the present study was to evaluate if pamidronate (PA), which was previously demonstrated to reduce spinal fusion rate when administered continuously for 8 weeks, would increase the spinal fusion rate if administered in a single dose at the time of surgery in a rabbit model. Thirty-two New Zealand rabbits underwent an L5–L6 posterolateral intertransverse fusion with iliac crest autograft. Animals were randomized to receive either PA 3 mg/kg in a single dose immediately after surgery, or normal saline. Animals were killed 8 weeks after surgery and fusion was determined by manual palpation and radiographic analysis. Fusion healing was obtained in eight rabbits (50%) in the PA group and in four animals (25%) in the control group, $p = 0.137$. In a rabbit model, a single dose of PA did not decrease lumbar spinal arthrodesis consolidation rates, but it obtained a nonsignificant higher spinal fusion rate.

Keywords Spinal fusion · Bisphosphonates · Animal model · Nonunion

Introduction

Posterolateral spinal fusion (PLF) is a surgery frequently performed to treat degenerative disorders, tumors, infections, trauma and deformities of the spine. Nonunion rates reported for PLF in the lumbar spine vary from 0 to 60% [9, 10, 14, 15, 20, 32, 39]. Factors that influence fusion healing include the disease for which the fusion was indicated, type of graft used, number of levels fused, fusion technique, presence of instrumentation, smoking habits, and drugs the patients receive [7, 10, 13, 17, 29, 34, 35]. PLF is usually done in older patients, who may be receiving treatment with bisphosphonates (BP), but limited data are available on BP action on spinal fusion.

The effect of BP on spinal fusion consolidation in human beings has not been determined yet. Few studies performed on animal models have evaluated the effect of BP on consolidation of spinal arthrodesis, and their results have been contradictory. Most of these studies have treated animals with BP for several weeks before or after a PLF, showing negative effects on spinal fusion consolidation, with dose-dependent lower fusion rates and slower graft incorporation [3, 4, 18, 21, 37]. In contrast, Bransford et al. [8] showed that a single dose of zoledronic acid (ZA) administered at the time of surgery resulted in a higher fusion rate (however, not reaching statistical significance) that was associated with an increase in bone mineral density. Such contrasting results in the aforementioned papers led us to question whether the differences in spinal fusion rates are due to the type of BP used or to the timing of BP administrations.

We hypothesize that pamidronate (PA), which was previously demonstrated to reduce fusion rate when administered continuously for 4 weeks preoperatively and 4 weeks postoperatively [3], would increase the spinal

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fusion rate if administered in a single dose at the time of surgery, as it was shown with a single dose of ZA in the Bransford study [8]. To perform the present study we used a validated rabbit model described by Boden [6].

Materials and methods

Institutional review board approval for animal research was obtained prior to performing this study.

Thirty-two New Zealand male rabbits (weighing between 2,070 and 3,350 g), underwent bilateral, intertransverse process, single level fusion at L5–L6, using autologous bone graft obtained from both iliac crests, as described by Boden et al. [6]. Animals were sedated with 0.2 mg/kg of acepromazine hydrochloride and 15 mg/kg i.m. solution of ketamine hydrochloride. General anesthesia was administered with an intravenous solution consisting of 20 mg/kg of ketamine hydrochloride plus 5 mg/kg of xylazine.

A bilateral posterolateral (intertransverse) spinal fusion was performed between L5 and L6. A standard posterior midline incision was performed through the subcutaneous tissues. Through bilateral, independent, intermuscular approaches, the transverse processes of L5 and L6 were exposed and decorticated with a high-speed circular burr. Autologous cortico-cancellous iliac crest bone graft was obtained through the same skin incision, but through a different fascial approach. In order to reduce the fusion rate described for this model (since a higher fusion rate makes it difficult to power a study to demonstrate a statistical difference), we chose to harvest a smaller graft volume, which was demonstrated to decrease fusion rates [12, 31]; the graft volume used was 1.5 cc per side. Bone graft was applied between the decorticated intertransverse processes. The fascia and the skin were both closed with absorbable 1-0 sutures.

Animals were randomized immediately after surgery, so that 16 rabbits received PA 3 mg/kg in a single dose immediately after surgery, or normal saline in the same volume (16 rabbits). The animals were coded and the administration of the drug was performed by personnel other than the surgeons and evaluators. The investigators did not know which group the animals belonged to.

Postoperative analgesia was obtained with subcutaneous Tramadol hydrochloride 4 mg/kg (Tramal[®], Grunenthal) every 12 h for 5 days and then every 24 h for 3 more days. Postoperative prophylactic subcutaneous Enrofloxacin (5 mg/kg) was used before surgery started and every 24 h for 5 days.

Animals were killed with an anesthetic overdose 8 weeks after surgery. The whole lumbar spine was dissected and soft tissues were removed to allow complete

visualization of L5–L6. The presence of fusion was analyzed with two different methods. A manual palpation test, as described by Boden [6], was applied by two independent observers blinded to the animal group; if the fused level moved as a block, it was considered solid. An anteroposterior X-ray was obtained from each specimen after removal from the animal; a fusion was considered consolidated if there was a continuous bony mass, crossing both transverse processes, without radiolucent lines on the radiograph (Fig. 1). For a specimen to be considered bearing a solid fusion, absence of movement on palpation test plus bone bridging on radiographs had to be demonstrated. The examiners were not aware during the whole evaluation stage if PA or saline solution had been administered.

The sample size estimation was based on data from a previous study by O'Loughlin et al. [31] who reported a fusion rate of 80% in the treated group and 30% in the control group. To obtain a similar difference, with a significance level of 5% and a power of 80%, the smallest appropriate sample size was 15 animals in each group.

The statistical analysis of the data obtained was carried out using the Fischer exact test, and the non-parametric test of Mann–Whitney. A $p < 0.05$ was considered to be a statistically significant difference.

Results

A total of 32 rabbits were studied, 16 in each group. The weight of the animals was similar in both groups; the median weight of the animals in the PA group was 2,651 g (2,070–3,350) and in the control group it was 2,613 g (2,340–3,180), $p = 0.72$.

Considering the absence of mobility in flexion and extension plus bone bridging among transverse processes on radiographs, fusion healing was noted in eight rabbits (50%) in the PA group and in four animals (25%) in the control group ($p = 0.137$). The median weights of the animals according to the state of consolidation were: 2,640 g for rabbits with nonunion, and 2,619 g for the animals with a solid fusion ($p = 0.92$).

Discussion

Bisphosphonates are widely used to treat a variety of bone diseases, mainly bone mineral loss (osteopenia and osteoporosis), but they may affect the bone healing in fractures and bone fusion. BP has an anti-catabolic effect on bone; its mechanisms of action include inhibition of osteoclast activity (by means of lowering cell recruitment and decreasing cell binding to bone), osteoclast inactivation

Fig. 1 Radiographs showing two specimens with intertransverse fusion (**a**), and two specimens with a nonunion (**b**)

and apoptosis, thus reducing bone resorption [17, 25]. Several studies using BP in long bone fractures and distraction osteogenesis in animal models have shown increased calcium content and callus volume [1, 5, 11, 16, 19, 22–24, 26–28, 33, 36]. Their anti-catabolic effect is associated, however, to slower bone remodeling, and a callus with diminished mechanical strength [22–24]. Recently, it has been suggested that the dose and frequency of administration of BP can influence this effect on callus formation, with a single dose use producing better results than a chronic administration [2, 36].

Previous studies that have evaluated the effect of BP on spinal fusion have been scant and have only been conducted on animal models. Most studies have evaluated the

effect of Alendronate administered for weeks before or after the animals undergo a spinal fusion, with a negative impact on the fusion rate [3, 18, 21, 37]. Recently, it was shown that PA administered continuously for 4 weeks preoperatively and then daily for 4 weeks postoperatively had a negative effect on a lumbar fusion rabbit model [3]. In contrast, a recent study from Bransford et al. [8], showed a 63% fusion rate using a single dose of ZA, compared to 25% in controls (without statistical significance). This effect might be explained by the single dose administration, or because ZA has a stronger biological effect than Alendronate or PA. The lower fusion rates associated with a continuous administration of BP in previous studies cannot be explained by caretaker handling of the animals,

as they have used control groups different to the study groups only in the drug administered.

We used a single dose of PA administered at the time of surgery, to evaluate if this PA administration would increase the fusion rate like in Bransford study, or if it would decrease the fusion rate as shown by Babat et al. [3], who used PA administered three times/week for 4 weeks preoperatively and then daily for 4 weeks postoperatively in the same rabbit model. Although we did not demonstrate a significant increase in fusion rate, the PA group exhibited a 50% consolidation, compared to 25% in the control group. Nonetheless, as our results are similar to what Bransford et al. [8] found with a single dose of ZA, we suggest that it is not the drug, but the dose and the way it is administered that affects the fusion rate; one dose of BP administered at the time of surgery could increase fusion rate, in contrast to what has been described for the chronic administration of BP. To elucidate this issue, future studies should compare groups with a continuous administration and a single dose administration of BP. In addition, Babat's study used a much higher dose (a total of 30 mg in 8 weeks) than that used in our protocol (3 mg/kg in a single dose). The dose we chose was based on data by Little et al. [1], which evidenced good results using such dose in long bone callus formation in animals. The recommended dose for human use is 0.5–1.5 mg/kg in every dose [25], and it is prescribed on a monthly or quarterly basis.

BP may be used in osteoporotic patients that undergo a spinal fusion not only to decrease the fracture risk associated with osteoporosis, but also to increase bone-screw fixation, as recent studies have shown that BP can improve screw fixation in osteoporotic long bones [30] and they also enhance bone-screw interface fixation in an experimental model in the spine [38].

A limitation of our study is that we did not perform histological or histomorphometric studies to evaluate the microscopic characteristics or the volume of the bone produced. We also have to address that our study used young rabbits, without osteoporosis; the results obtained might be different using osteoporotic animals. However, the major limitation of our study is that the lack of statistical difference may be secondary to a type II error; the minimal number to demonstrate a significant difference based on the fusion rates observed would be 28 rabbits in each group.

Our study shows that in an animal model, a single dose of PA does not decrease lumbar spinal arthrodesis consolidation rates, and it can produce a nonsignificant higher spinal fusion rate. Future studies should verify if these results are also observed in patients that undergo a spinal fusion.

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