A shape memory alloy implant can be an effective surgical treatment in the atlantoaxial joint stabilization using rabbits as substitutes for toy-breed dogs

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Received July 11, 2023
Accepted August 3, 2023
doi.org/10.2460/ajvr.23.07.0158

OBJECTIVE
To investigate the feasibility of using shape memory alloy (SMA) implants for atlantoaxial joint stabilization using a rabbit model as a substitute for canines.

ANIMALS
20 rabbit cadavers.

METHODS
We prepared rabbit cadavers from the middle of the skull to the third cervical vertebra. The vertebral body and canal sizes of the atlas and axis were compared using CT data from rabbits, normal dogs, and dogs with atlantoaxial instability (AAI) to assess the feasibility of using rabbits as substitutes for toy-breed dogs. The shape memory alloy (SMA) implants were designed to stabilize the atlantoaxial joint without compromising the spinal canal passage for safety and were classified into SMA-1 and SMA-2 based on their design. To evaluate the strength, the ventrodorsal force was measured with atlantoaxial ligaments intact, after removing the ligaments, and after applying conventional wire or SMA implants to stabilize the atlantoaxial joint. The time taken for implant application was measured.

RESULTS
No significant difference in vertebral body size of the atlas and axis was observed. A significant difference in vertebral canal size was observed between the animals. In biomechanical testing, the SMA-2 implant provided more stabilization, while the SMA-1 implant had lower strength than the conventional method using wires. The application time of wire was the longest, while that of SMA-1 was the shortest.

CLINICAL RELEVANCE
SMA implants provide comparable strength and demonstrate superior efficacy compared to conventional dorsal wire fixation of atlantoaxial stabilization. Therefore, SMA implants can be an effective surgical option for AAI.

Keywords: shape memory alloy, shape memory effect, atlantoaxial instability, dorsal stabilization, rabbit cadaver
epidural space of the vertebral canal. Nevertheless, several studies have shown that patients with cranio-cervical junction and vertebral malformations as well as AAI are more suitable for the dorsal fixation technique than the ventral fixation technique because of the difficulty of application. A new dorsal fixation technique using the Kishigami tension band has been reported as a substitute for previous dorsal fixation in AAI. However, the technique was not commercially available and posed difficulties in fixing the wire after applying the Kishigami tension band. Therefore, an easier and safer dorsal fixation technique is needed.

Many studies on shape memory alloy (SMA) implants exist in human medicine, especially AAI, traumatic cervical instability, and degenerative lumbar spinal surgery. SMAs are alloys composed of nickel and titanium that possess characteristics such as superelasticity, long fatigue life, and high corrosion resistance. Due to these mechanical properties, SMAs exhibit improved biomimetic nature compared to traditional implant materials in neurosurgery. In addition, these materials have a special ability of shape memory effect (SME), which can change their shape at temperatures below 10 °C and return to their original shape at temperatures above 30 °C, while retaining rigidity. Currently, SMAs are used in dynamic spinal stabilization due to their functional properties, known to offer simpler and less invasive insertion compared to other surgical treatments. We sought to use SMA implants for dorsal stabilization in AAI, leveraging their unique characteristics.

In veterinary medicine, biomechanical tests have mainly been conducted using beagle cadavers to evaluate strengths after the development of new implants for AAI. However, the use of beagle cadavers has been limited in AAI research because AAI primarily occurs in small-breed dogs. Therefore, a suitable alternative animal model such as small-breed dogs is necessary. This study aimed to (1) establish a rationale for using rabbits as an alternative to toy-breed dogs by comparing the size of their vertebrae, and (2) introduce and develop a novel dorsal fixation technique using SMA to stabilize the atlantoaxial joint. We hypothesized that a new dorsal fixation method with SMA using the SME might be more effective and safer than dorsal fixation with a wire in AAI. We evaluated the ventrodorsal load through biomechanical testing and described the differences between SMA implants and previously used methods.

Methods

Study subjects

This study used rabbit cadavers (n = 20, New Zealand White) euthanized for reasons unrelated to the study. The study protocol was approved by the Institutional Animal Care and Use Committee (SNU-210303-7-2). The 2 studies were conducted using rabbit cadavers.

To compare the sizes of the vertebrae and vertebral canals, this study included 5 rabbit cadavers, 5 normal dogs, and 5 dogs with AAI. The 5 rabbit cadavers were randomly selected from the rabbit cadavers used in the study. The dogs (n = 10) were divided into 2 groups: those without cervical disease (5) and those with AAI (5). Dogs weighing > 5 kg were excluded from the analysis. Patients with diseases related to the cervical spine were excluded from the normal dog group. Inclusion criteria of dogs in the AAI group were dogs with AAI diagnosed using CT and magnetic resonance images. The mean age was 24 weeks, and the mean body weight was 3.3 kg (range, 2.9 to 3.59 kg) in the rabbit group. The mean age was 7.6 years (range, 4 to 12 years), and the mean body weight was 3.4 kg in the normal dog group. The mean age was 54.6 months (range, 9 to 96 months), and the mean body weight was 2.53 kg (range, 2.1 to 3.25 kg) in dogs in the AAI group.

We used 20 rabbit cadavers to compare novel dorsal fixation using SMA implants and conventional wire dorsal fixation for AAI stabilization. These cadavers were covered with saline-soaked gauze and stored at −20 °C. The cadavers were thawed at room temperature for 2 days before testing. We limited all cadavers to no more than 3 freeze-thaw cycles in total. Before testing, the samples were prepared by performing a dorsal midline incision. The dorsal muscles were removed to reveal the atlas (C1) and axis (C2), whereas the C1 and C2 ventral muscles (longus colli muscles) were maintained to avoid separation of C1 and C2. Sample segments were prepared from the middle of the skull to the third cervical vertebra using a saw. Before testing, the samples were stored in saline-soaked gauze to avoid drying.

Measurement of the size of vertebral bodies and vertebral canal

We included 3 groups in this study: rabbits, normal dogs, and dogs with AAI. CT scans were performed in these groups, the dogs (n = 10) underwent CT scans for reasons unrelated to the current study. We obtained sagittal, transverse, and dorsal images in the form of DICOM files. RadiAnt DICOM viewer software (version 1.9.16.7446; 64-bit; Medixant) was used to compare the size of the vertebral bodies and vertebral canal and to create multiplanar reconstructions. The evaluation criteria used to compare the atlas and axis among rabbits, normal dogs, and dogs with AAI included the following parameters: vertebral body width (VBW), vertebral body depth (VBD), vertebral canal width (VCW), vertebral canal depth (VCD), and lengths for SMA implant fabrication. The vertebral body and canal size of the atlas were measured from the largest part of the vertebral canal in the transverse plane. The VBW was measured from one end of the atlas wing to the other, and the VBD was measured from the dorsal tubercle to the ventral tubercle in C1 (Figure 1). In the axis, the measurements were taken in the middle of the vertebral body in the sagittal plane. The VBW was measured from the left transverse process to the right transverse process, and VBD was measured from the tip of the spinous process to the body in the middle of C2 in the sagittal view. The VCW and VCD of C1 and C2 represent the length of vertebral canal width and depth,
respectively. To determine the length required for the fabrication of SMA implants, the distance between the grooves on both sides of C1 was measured and is represented. In addition, the lengths from C1 to C2 were measured and are represented.

Design and fabrication of shape-memory alloy implants

In this study, we focused on the design and development of SMA implants for the stabilization of the atlantoaxial joint, considering their efficacy, safety, and stabilization aspects. The unique property of SMA, known as SME, was used in the design of these implants. The SME could be magnified in terms of efficacy because the SMA implant could return to its original shape at the proper temperature. To ensure safety, the SMA implants were designed not to pass through the epidural space of the vertebral canal, unlike conventional dorsal fixation with wire. The first SMA model (SMA-1) was designed to hook the cranial part of the implant to the dorsal atlanto-occipital region, and the caudal part of the implant was hung caudal to the spinous process (Figure 2). To enhance the stabilization, a second SMA model (SMA-2) was developed, which included additional hooks on the caudal part that engaged with the holes in the spinous process.

A 0.5-mm-slice CT scan of the cervical vertebral column was acquired from each specimen to manufacture case-specific SMA implants. The 3-D virtual vertebral models were created using software (Mimics 20.0; Materialise), and the SMA implant models were designed using a 3-D modeling software (Solidworks 19.0; Dassault Systems SA). In the design process of SMA-1, the length between both side grooves at the point of the dorsal tubercle in C1 (Figure 1) and the length from the C1 body to the C2 body were required. For the SMA-2, the lengths from the C1 body to the cranial and caudal holes of the spinous process were required. The positions of the spinous process holes were determined based on the CT data, with holes placed at the thickest part of the spinous process. During the fabrication of the SMA implants, the length of the implants was set to 80% of the real length. The thickness of the SMA implants was set at 0.8 mm similar to the thickness of wire (0.8 mm). The SMA implants were fabricated by melting a Ti-Ni alloy using a high-frequency induction melting method. The molten alloy was poured into a mold with a diameter of 20 mm to form an ingot. Subsequently, the ingot was rolled to produce a 0.8-mm SMA wound around the jig to create the desired implant shape.

Surgical technique

We used rabbit cadavers (n = 20) in biomechanical testing to compare the strength of each technique, including dorsal stabilization using wire and the new technique with SMA-1 and SMA-2 implants. There were 5 groups: intact, defect, wire, SMA-1, and SMA-2. The intact and defect groups included rabbit cadavers in which the ligament-stabilized atlantoaxial joint was intact and removed, respectively. The defect group underwent biomechanical testing after the intact group, following complete ligament removal. The wire, SMA-1, and SMA-2 groups included rabbit cadavers in which the atlantoaxial joint was stabilized using wire, SMA-1, and SMA-2, respectively.

In wire fixation, a stainless-steel wire (0.8 mm) was applied as previously described.24 The dorsal atlanto-occipital fascia was carefully incised, and the stainless steel wire was passed cranially under the dorsal arch of the atlas. The loop was folded back toward the axis, cut to insert holes of the spinous process, and threaded.
In SMA-1 and SMA-2 fixation, a temperature controller was used to lengthen the implants. The dorsal atlanto-occipital membrane was then carefully incised. The implants were lengthened with mosquito forceps at a low temperature (4 °C), and the cranial hook of the SMA implants was applied under the dorsal lamina of the atlas. The caudal part of the implants was fixed within the spinous process of C2 in SMA-1. For SMA-2, the caudal part of the implant was inserted in the predrilled holes in the spinous process. After applying SMA-1 and SMA-2, hot saline (30 to 35 °C) was applied to implants to return to their original shapes. The testing was applied with a heating machine to maintain up to a certain temperature (30 to 36 °C) in SMA groups. The application time of the implants was recorded for each group. The application time was measured only by the time taken for implant placement, excluding the time required for muscle dissection and making holes in the spinous process.

Mechanical testing
Each specimen was videotaped during testing. Mechanical testing was conducted using mechanical machines (MTS Bionix Tabletop Test System [MTS System Corp]; Instron E3000 [Instron]). Each specimen was placed in a mechanical machine at a distance of 20 mm between 2 support pins. The position of the load pin was set in the atlantoaxial joint and the testing started when the load pin contacted the atlantoaxial joint of the specimen. Ten cycles were performed in the ventrodorsal direction for each case, with the first cycle applying a load of 16 N. The ventrodorsal force load was measured at the AAI point. The results of each cycle were recorded, and the curve pattern was compared with the point of atlantoaxial instability observed on the videotape. During the cycles, additional measurements were not performed in case of fracture of the atlas or axis.

Statistical analysis
Data were analyzed using GraphPad Prism (version 9.5.1). The normality test was conducted using the Shapiro-Wilk test, and statistical analysis was performed using 1-way ANOVA due to the normal distribution observed in all the data. One-way ANOVA was used to compare the size data of the vertebral bodies and vertebral canals among rabbits, normal dogs, and dogs with AAI. In cases where statistically significant ANOVA differences (P < .05) were observed, post hoc testing was conducted using the Bonferroni correction to account for multiple comparisons. Statistical significance for comparison of vertebral bodies and canal size was set at P < .05. In addition, 1-way ANOVA was performed to compare (1) the ventrodorsal force among the defect, intact, wire, SMA-1, and SMA-2 groups, and (2) the application time among the wire, SMA-1, and SMA-2 groups. Similar to the previous analysis, post hoc testing with Bonferroni correction was used for multiple comparisons when statistically significant ANOVA differences (P < .01) were detected. Statistical significance for the comparison of ventrodorsal force and application time was set at P < .01.

Results
Comparison of atlas and axis in the rabbits, normal dogs, and dogs with AAI
There were no significant differences in the VBW and VBD of C1 and C2 between groups (Figure 3).
Figure 3—Comparing the size of vertebral body and vertebral canal in rabbit, normal dog, dog with atlantoaxial instability. The size of the vertebral bodies in C1 and C2 was compared in each group (A). The size of the vertebral canal was compared in each group (B). The distance between both side grooves in C1 (C) and the length from the C1 to C2 (D + E) were compared in each group (D). *Significant differences between groups ($P < .05$).

Table 1—Lengths of vertebral bodies and vertebral canal in C1 and C2.

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<th>C2 (cm)</th>
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<th>Vertebral canal of C2 (mm)</th>
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However, a significant difference was observed in the size of the vertebral canal between the rabbit group and the other groups. The VCW and VCD of C1 and C2 in the rabbit group were smaller than those in the normal dog group and dogs with AAI group (P < .05). Comparing to the distance between the grooves on both sides of C1 and the length from C1 to C2, there was no significant difference among the groups. The data represent the size of the vertebral body and vertebral canal in C1 and C2, respectively (Table 1).

Biomechanical testing
A significant difference was observed in the defect group compared to intact groups (P < .01) (Figure 4). In addition, there were significant differences between the intact group and the other groups (P < .01). The ventrodorsal forces of the groups with implants for stabilization of the atlantoaxial joint were higher than that of the intact group. The average ventrodorsal force measured was 57.49 ± 2.78 N, 77.66 ± 6.69 N, and 75.61 ± 4.26 N in the SMA-1, SMA-2, and wire groups, respectively. Among the stabilization groups, the SMA-2 group exhibited the highest mean ventrodorsal force.

Application time
A significant difference was observed in the wire group compared to the SMA groups (P < .01) (Table 2). The wire group required a significantly longer application time than the SMA groups. In contrast, the SMA-1 group had the shortest application time. The application time in the SMA-2 group was approximately 3 times longer than in the SMA-1 group. Furthermore, the application time in the wire group was approximately 4 times longer than that in the SMA-2 group.

Discussion
In veterinary medicine, previous studies used beagle cadavers for neurosurgery research. However, congenital diseases such as AAI commonly occur in small-breed dogs. Therefore, it is necessary to develop alternative animal models that possess anatomical characteristics and vertebral body size similar to those of small-breed dogs. In this study, we aimed to compare the size of the vertebral body and vertebral canal between toy-breed dogs with, or without AAI, and rabbits using CT data. Our findings revealed no significant difference in the size of the vertebral bodies of C1 and C2 between the rabbit and other groups. However, there was a significant difference in the vertebral canals of C1 and C2 between the rabbit group and other groups. Although the size of C1 and C2 vertebral canals in the rabbit group was smaller than that in the other groups, the most important factor for applying implants for atlantoaxial stabilization is the size of the vertebral body and not the vertebral canal. Based on these considerations, we concluded that rabbit cadavers could be suitable substitutes for small-breed dogs in studies focused on stabilizing the atlantoaxial joint. This study represents the first step in the use of rabbits as a substitute for small-breed dogs in AAI research, thus fulfilling the requirement for appropriate animal models.

It has been reported that dorsal fixation methods are generally easier than ventral fixation for AAI. However, dorsal fixation methods for AAI have several limitations, such as the risk of iatrogenic spinal cord injury, vertebral artery injury, fractures, and even death. For example, dorsal fixation with wire can lead to iatrogenic spinal cord injury and spinous process fractures, while dorsal fixation using...
suture materials is easier but relatively weaker than dorsal fixation with wire. In an attempt to address these issues, the Kishigami tension band technique was introduced. However, it is not commercially available and inconvenient to use wire as the next step after applying the Kishigami tension band. Therefore, an ideal dorsal fixation method should aim to (1) reduce complications such as iatrogenic injury, (2) be easier to apply with a short operation time, and (3) have strengths comparable to conventional dorsal fixation methods. Therefore, we developed a dorsal fixation technique using the SMA implants to stabilize the atlantoaxial joint.

In human medicine, SMAs are used for safe and effective fixation and stabilization in neurosurgery. SMA implants can be custom-made for each patient, considering their unique size and shape. In addition, SMAs are known for less invasive insertion using SME effects than other surgical treatments. Based on these advantages, we developed patient-specific SMA implants for AAI surgery in veterinary medicine. Our primary focus during the developmental process was to minimize the risk of spinal cord damage. To achieve this, we designed the SMA implants to avoid passing through the epidural space of the vertebral canal. The first SMA implant model was designed as a loop, which is used in human neurosurgery to ensure ease of application and enhance safety. To reinforce the stabilization, the second model of the SMA implant was made to be fixed to the spinous process by adding 2 holes to the spinous process. Ultimately, we believe that the developed SMA implants offer improved safety compared to conventional dorsal fixation methods that involved implant placement through the spinous canal.

SMAs are easily applied compared to other surgical treatments in neurosurgery due to SME properties. We considered that the ability of SME could provide easier and more effective application of the implants. For convenience, the application time of the different implants (wire, SMA-1, and SMA-2) was measured. The application time for the SMA-1 implant was shorter than that for the other groups because the SMA-1 implant was not required to pass through the holes in the spinous process. Although the SMA-2 implant passed through the holes in the spinous process similar to the wire implant, its application time was 4 times shorter than that of the wire implant. These findings are similar to those of human studies that showed that SMAs have easy application and short operating time due to their SME properties in neurosurgery. We believe that the difference in application time was due to (1) the SME ability, which memorized the original shapes; and (2) not passing the implant through the epidural space. For convenience, the SMA implants showed better results than the wire implants.

In human medicine, stabilization of SMA implants was proven through biomechanical testing. We evaluated the strength related to flexion of the different implants (wire, SMA-1, and SMA-2) because the fixation technique for AAI should have a high resistance of flexion, thus preventing dorsal subluxation of the axis. All implant groups exhibited higher ventrodorsal force than the intact group. These results showed that all implant groups provided more stability to the atlantoaxial joint than the intact group. The ventrodorsal force in the SMA-2 group was greater than that in the wire group; however, there was no significant difference between the SMA-2 and wire groups. The SMA-1 group showed less stabilization than the wire group. Indeed, there was a report on combining ventral stabilization with SMA loop implants in AAI surgery to provide more structural stability. Therefore, we consider that the SMA-1 implant may be suitable when used in conjunction with ventral stabilization techniques, as it may not provide sufficient stabilization with only the SMA-1 implant. In contrast, SMA-2 implants can be an effective surgical option for AAI because they have comparable strength to wire implants with easier and safer application.

This study had several limitations. First, the number of specimens was small. Second, the ventrodorsal force of ligaments in rabbit cadavers, which stabilizes the atlantoaxial joint, was not analyzed compared to those of toy-breed dogs. However, the stability of the SMA implant was ensured by comparing the biomechanical properties of the specimens after the stabilization of the atlantoaxial joint using a previously reported conventional method. Third, SMA has advantages including long fatigue life, high corrosion resistance, and biocompatibility. However, these features were not evaluated in this study because of the use of cadavers. Thus, further studies on corrosion resistance and biocompatibility are necessary.

In conclusion, this study showed that rabbit cadavers can serve as substitutes for small-breed dogs in AAI research. In addition, SMA implants showed several beneficial aspects of convenience, safety, and stabilization compared to the conventional method of wire. Thus, we suggest that the use of SMA implants is an effective surgical option for the stabilization of the atlantoaxial joint.

Acknowledgments

Author contributions: YS—Study conception and design, conducting experiment, acquisition, and interpretation of data, drafting of the manuscript, and approval of the final article; BJK—Study conception and design, critical revision of the manuscript, and approval of the final article; SKC—Conducting experiments, acquisition, and interpretation of data; WKK—Study conception and design, critical revision of the manuscript, and approval of the final article. All authors contributed to editing the manuscript and approved the submitted version.

We would like to thank Editage (www.editage.co.kr) for English language editing.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

Funding

The authors have nothing to disclose.
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