Application of fiber tractography and diffusion tensor imaging to evaluate spinal cord diseases in dogs

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ABSTRACT. Fiber tractography is a technique capable of depicting the three-dimensional structure and connectivity of nerve fibers using serial magnetic resonance diffusion tensor imaging (DTI). To establish fiber tractography and DTI methods in veterinary clinical medicine, we evaluated fiber tractography and DTI parameters: apparent diffusion coefficient (ADC) values and fractional anisotropy (FA) values, in various spinal cord diseases. Spinal cord DTI was examined in 28 dogs with spinal cord diseases. The ADC and FA values were measured at lesion sites and cranial normal sites on spinal cords, and both values of lesion sites were compared with normal sites. In thoracolumbar intervertebral disk herniation (IVDH) cases, depending on their neurologic grades, fiber tractography indicated rupture of fiber trajectories, loss of neuronal bundles and disorder of fiber directions. In these cases, the average ADC values at lesion sites significantly decreased compared with normal sites (P=0.016). In the progressive myelomalacia case, the average ADC and FA values of hyperintense swollen regions in T2WI decreased compared to both values in other disease cases. Finally, in the meningioma case, the continuity of fiber trajectories improved after the administration of an anticancer agent. This study suggests that fiber tractography and DTI are useful in the diagnosis and prognosis of veterinary spinal cord diseases.

KEY WORDS: diffusion tensor imaging, dog, fiber tractography, MRI, spinal cord disease

Recently, magnetic resonance imaging (MRI) has become widely used to examine spinal cord diseases, such as intervertebral disk herniation (IVDH), in veterinary medicine. However, it is difficult to definitively diagnose degeneration diseases, such as degenerative myelopathy (DM), or to predict the prognosis of spinal cord diseases, such as those progressing from IVDH to progressive myelomalacia (PM), by conventional MRI [5, 16]. PM refers to the ischemic and/or hemorrhagic necrosis of the spinal cord that can occur following an acute severe spinal cord injury, such as IVDH [18]. In these spinal cord diseases, conventional MRI cannot detect functional continuity and degeneration of the spinal cord nerve fibers. Therefore, it is necessary to evaluate the functional continuity of the nerve fibers in order to make a definitive diagnosis and a prognostication of these diseases.

Among MRI techniques, fiber tractography is more likely to detect the continuity of nerve fibers, because it can depict the three-dimensional structure and connectivity of nerve fibers using serial diffusion tensor imaging (DTI) [14]. Fiber tractography is based on the concept that the direction of fast water diffusion is indicative of the overall orientation of the neuronal fiber [3, 13]. It has been shown that this technique can evaluate the degree of integrity and connectivity of neuronal bundles, even though it may not represent accurate neuronal fibers themselves [2, 3]. Representative DTI parameters include apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values: ADC indicates the magnitude of the tissue water diffusion, regardless of its direction [15], while FA indicates the anisotropic strength of the diffusion [4]. The ADC and FA values have been used to detect abnormal neuronal tissue [7, 22]. Fiber tractography and measurements of ADC and FA values may be used to evaluate the functional connectivity of spinal cord that is undetectable through conventional MRI.

In this study, for the clinical application of fiber tractography and DTI methods to spinal cord diseases in veterinary medicine, we imaged DTI with a 12-direction motion probing gradient (MPG) using 3.0 T MRI and evaluated ADC and FA values in various spinal cord diseases. The continuity of spinal cords in dogs affected by neurologic diseases was evaluated using fiber tractography.
MATERIALS AND METHODS

Animals

All procedures were approved by The Animal Ethics Committee, Faculty of Agriculture, the University of Miyazaki (approved number: 2015–07-31–213). Study subjects included 28 dogs with neurologic diseases that underwent MRI examination at the University of Miyazaki Veterinary Teaching Hospital from May 2013 through May 2016. The breeds of these dogs with thoracolumbar IVDH (n=23) were as follows: Miniature Dachshund (n=14), French Bulldog (n=2), Maltese (n=2), Cavalier King Charles Spaniel (n=1), Miniature Pinscher (n=1), Pug (n=1), Toy Poodle (n=1) and Welsh Corgi Pembroke (n=1). The breeds of these dogs with cervical IVDH (n=3) were as follows: Beagle (n=1), Shiba Inu (n=1) and mongrel (n=1). The breed of a dog with PM (n=1) was Miniature Dachshund (n=1). The breed of a dog with meningioma (n=1) was mongrel (n=1). The average age of subjects was 7 years (2–13 years), and the study included 15 males and 13 females. Thoracolumbar IVDH cases were graded from 1 to 5, based on the severity of the neurologic dysfunction by neurologic examination, as follows: grade 1 (n=2) indicated thoracolumbar pain only, with no neurological deficits; grade 2 (n=7) indicated an ambulatory paraparesis case; grade 3 (n=7) indicated a non-ambulatory paraparesis case; grade 4 (n=4) indicated paraplegia with positive deep pain sensation; and grade 5 (n=3) indicated paraplegia with a loss of deep pain sensation [1, 19].

MRI examinations

All MRI examinations were performed by a 3.0 T MRI scanner (Vantage Titan 3T™, Toshiba Medical Systems, Tokyo, Japan). For MRI procedures, general anesthesia was induced with intravenous propofol (PROPOFOL injection, Fuji Pharma Co., Ltd., Toyama, Japan) and, following orotracheal intubation, anesthesia was maintained with a mixture of isoflurane (ISOFLU®, DS Pharma Animal Health Co., Ltd., Osaka, Japan) in oxygen and medical air. Cervical spinal cord imaging was performed in the prone position with a shoulder coil or in the dorsal position with a head coil. Thoracolumbar spinal cord imaging was performed in the dorsal position with a spine coil and a 4ch flexible coil. Sagittal and transverse T1-weighted imaging (T1WI) and T2-weighted imaging (T2WI) were acquired by fast spin echo (FSE) pulse sequence. For sagittal imaging, the imaging parameters were as follows: repetition time (TR)=670/echo time (TE)=12 msec (T1WI) and 5,000/108 msec (T2WI), field of view (FOV)=20–25 × 20 cm, slice thickness=2 mm, gap=0.4 mm and number of excitations (NE)=2. Transverse DTI data were acquired using pulsed sequence: single-shot, spin-echo, echo-planar imaging (SE-EPI). The diffusion-weighting gradient schemes with 12 non-collinear directions were used based on our pilot study. The respiratory-gated imaging program was used in thorax and upper lumbar spine cases (TR=4,004–6,866 msec without respirator-gating or 9,000–9,478 msec with respirator-gating, TE=90 msec, slice thickness=2 mm, matrix=256 × 96, FOV=13 × 13.5 cm and NE=3, b value=1,000 mm²/s).

Image analysis

Fiber tractography, ADC maps and FA maps were created using the DTI package built into the MRI scanner. In determining the fiber tracts, we set the threshold of FA to 0.15 and the angle between two consecutive steps at greater than 45 degrees. Seed regions of interest (ROIs) were set in two regions, specifically the cranial and caudal sides of the lesion site, on the spinal cord in transverse FA maps. Fiber trajectories were color coded: cranial-caudal trajectories were blue, dorsal-ventral trajectories were green, and right-left trajectories were red. The average ADC and FA values were measured by setting ROIs that did not include cerebral spinal fluid as much as possible on the spinal cord of transverse plane in each map by radiologists (Y. K. and H. S). In IVDH and meningioma cases, the ADC and FA values were measured at lesion and cranial sites with normal findings in T1WI and T2WI. In the PM case, the ADC and FA values were measured at the IVDH area as lesion site and at the cranial area as cranial normal site, because normal spinal cord sites were not detected in conventional MRI.

Statistical analysis

In IVDH cases, Wilcoxon signed-rank test was used to compare ADC and FA values between lesion and normal sites. Spearman’s rank correlation coefficient was used to examine the correlation between the neurologic grade of thoracolumbar IVDH and ADC values, as well as the neurologic grade and FA values. Statistical significance was set at P<0.05.

RESULTS

Thoracolumbar IVDH

In the current study, fiber tractography visualized the bent transformation by the excrescence to the vertebral foramen (Fig. 1). It also showed the discontinuity of fiber trajectories, such as the rupture of fiber trajectories, loss of neuronal bundles and disorder of fiber directions (Fig. 1). Depending on neurologic grade, the discontinuity of fiber trajectories became severe. The average ADC values of lesion (compression) sites and normal sites (mean ± SD) were 1.041 ± 0.386 × 10⁻³ mm²/s and 1.205 ± 0.240 × 10⁻³ mm²/s, respectively (Table 1). The average ADC values of lesion sites were significantly lower than those of normal sites (P=0.016). The average FA values of lesion and normal sites were 0.572 ± 0.160 and 0.542 ± 0.127, respectively (Table 1). The average FA values of lesion and normal sites were not statistically different (P=0.202). Additionally, there was no correlation between neurologic grade and ADC (P=0.400) and FA values (P=0.247) in lesion sites. Similarly, in normal sites, there was no correlation between neurologic grade and ADC (P=0.128) and FA values (P=0.267).
Fiber tractography showed that the cervical spinal cords were compressed by the intervertebral disk that deviated into the vertebral foramen; this was similar to the fiber tractography of thoracolumbar IVDH cases. However, fiber trajectories in cervical IVDH cases maintained continuity (Fig. 2). The average ADC values of lesion (compression) and normal sites were $0.961 \pm 0.118 \times 10^{-3}$ mm$^2$/s and $1.135 \pm 0.233 \times 10^{-3}$ mm$^2$/s, respectively. The average FA values of lesion and normal sites were $0.563 \pm 0.928$ and $0.542 \pm 0.118$, respectively (Table 2). The average ADC values of lesion sites tended to decrease, and the FA values of lesion sites tended to increase compared with each value of normal sites in a manner similar to thoracolumbar IVDH cases.

**Progressive myelomalacia (PM)**

This PM case was associated with IVDH. Fiber tractography showed short fiber trajectories around only two setting seed ROIs (Fig. 3 arrows). These trajectories lacked continuity in whole spinal cord regions, and T2WI visualized hyperintense swollen regions. In this case, the ADC and FA values were measured at the IVDH area as lesion site and at the cranial area as normal site. The average ADC and FA values of the lesion site (IVDH area) were $0.507 \times 10^{-3}$ mm$^2$/s and 0.587, respectively. The average ADC and FA values of the normal site (cranial site from IVDH area) were $0.294 \times 10^{-3}$ mm$^2$/s and 0.315, respectively. The average ADC and FA values of the normal site were lower than both average values of other disease cases (Table 2).

**Meningioma**

In the meningioma case, the tumor was removed surgically, but tumor recurrence was confirmed by MRI examination after two months from surgery. Therefore, an anticancer agent (NIDRAN® FOR INJECTION 25 mg, Daiichi Sankyo Co., Ltd., Tokyo, Japan) was administered to the patient four times every 6–8 weeks. Baseline MRI examinations were performed prior to the
administration of the anticancer agent and followed at six months post-administration in order to evaluate its therapeutic efficacy. After administration of the anticancer agent, the clinical condition of this case was improved. However, in T1WI and T2WI, MRI examinations did not detect a reduction of tumor size relative to pre-chemotherapy with the anticancer agent. Interestingly, fiber tractography was able to depict the improvement of a number of fiber trajectories and their continuity (Fig. 4). The average ADC and FA values of lesion sites were 0.696 × 10^{-3} \text{mm}^2/\text{s} and 0.558, respectively. The average ADC and FA values of normal sites were 0.978 × 10^{-3} \text{mm}^2/\text{s} and 0.593, respectively (Table 2).

**DISCUSSION**

The current study evaluated the functional continuity of spinal cord nerve fibers in various spinal cord diseases using fiber tractography and measurement of ADC and FA values. In the compression diseases, such as IVDH, that the spinal cord was bent by...
the pressing of an excrescence on the vertebral foramen, fiber tractography visualized the bent transformation caused by external pressure. In thoracolumbar IVDH cases that we were able to evaluate statistically, the average ADC values at lesion sites were lower than those at normal sites, but the average FA values could not detect the difference between lesion and normal sites. The average ADC and FA values of the normal (non-compression) site in the PM case were considerably lower compared with normal sites in other disease cases.

Fiber tractography is based on the concept that the direction of fast water diffusion is indicative of the overall orientation of nerve fibers [3, 13]. This technique can be used to reflect the degree of integrity and connectivity of neuronal bundles, although it may not accurately represent neuronal fibers themselves [2, 3]. Ruptured fiber tractography does not mean a disruption of neuronal fibers themselves, but a remarkable inhibition of water diffusion in the nerve fiber axon. In one study, Asanuma et al. reported that fiber tractography was able to visualize fiber trajectories in tumor-bearing rat brains, where myelin and nerve fibers were found to remain intact in edematous regions [2, 3]. In this study, fiber tractography in various spinal cord diseases indicated bending by external pressure, rupture of fiber trajectories, loss of neuronal bundles and disorder of fiber directions. Thus, fiber tractography may reflect the state and severity of spinal cord injuries.

In this study, the average ADC and FA values were compared in the same dog. In human medicine, many ADC and FA values of normal brain and spinal cord in various age groups have been reported [9, 11, 21]. In veterinary medicine, a few studies have reported ADC and FA values of normal brain and spinal cord in dogs [8, 10, 17]. Pease and Miller reported that the average ADC and FA values (mean ± SE) of cervical spinal cord in eleven normal Beagle dogs were 1.0253 ± 0.034 × 10⁻³ mm²/s and 0.72043 ± 0.0126, respectively [17]. Hobert et al. reported the average ADC and FA values (mean ± SD) of cervical spinal cord were 0.560 ± 0.0126 × 10⁻³ mm²/s (range from 0.217 to 0.857 × 10⁻³ mm²/s) and 0.447 ± 0.0163 (range from 0.291 to 0.631), respectively [10]. In our data on one Beagle dog, the average ADC and FA values (mean ± SD) of cervical spinal cord were 0.978 ± 0.170 × 10⁻³ mm²/s (range from 0.708 to 1.159 × 10⁻³ mm²/s) and 0.656 ± 0.078 (range from 0.538 to 0.742), respectively. The average ADC and FA values of thoracolumbar spinal cord were 1.206 ± 0.347 × 10⁻³ mm²/s (range from 0.947 to 2.053 × 10⁻³ mm²/s) and 0.573 ± 0.160 (range from 0.281 to 0.739), respectively. Thus, each value varied with breeds and sites of spinal cord in the normal dogs. It seemed difficult to compare each value among different dogs. Therefore, in this study, the ADC and FA values were not compared between abnormal and normal dogs, and the ADC and FA values were compared between lesion and normal sites in the same dog.

In thoracolumbar IVDH cases, fiber tractography indicated that the discontinuity of fiber trajectories was closely associated with neurologic grade. Furthermore, functional connectivity of the spinal cord and fiber tractography seem to correlate. In these cases, the average ADC values of lesion sites were significantly lower compared to those of normal sites (P=0.016). Meanwhile, the average FA values of lesion sites and cranial sites were not statistically different (P=0.202), but the average FA values of lesion sites showed a tendency to be slightly higher than those of normal sites. Generally, at compression sites without spinal cord injuries, such as edema and demyelination, the ADC values decrease and the FA values increase, whereas at compression sites with the same injuries, the ADC values increase and the FA values decrease [6]. The presence of edema and demyelination at spinal cord compression sites due to IVDH causes the ADC and FA values to fluctuate. It is a well-known fact that a change in tissue water content due to edema and lipid content due to demyelination greatly influences the MRI signal. Thus, the ADC and FA values were considered to be impacted by edema and demyelination. In contrast, fiber tractography could not be influenced by edema and demyelination. Fiber tractography is reportedly able to visualize fiber trajectories in edematous regions [2, 3], thereby, it might be useful in evaluating the severity of injury in the spinal cord.

The PM case in this study was not performed by histopathologic examination. This case was diagnosed from characteristic clinical signs and image findings suggesting PM [16, 18]. The chief complaint in this PM case was sudden paraplegia. Furthermore, there was progressive loss of pelvic limbs reflexes, and the level of panniculus cut-off moved cranially. The period...
from onset to MRI examination was 11 days. In MRI examination, IVDH (L1-2) was detected, and T2WI visualized hyperintense swollen regions between T12 and L5. Based on these findings, we diagnosed this case as PM. In the PM case, the average ADC and FA values of hyperintense swollen regions in T2WI were low compared to both average values in other disease cases. Softening and/or necrosis of the spinal cord are suspected to induce an increase in ADC values and a decrease in FA values in order to cause random water diffusion in nerve fibers. However, the average ADC value of hyperintense regions in T2WI decreased. This seems to be attributable to a widely cytotoxic edema and hemorrhage. In the brain, cytotoxic edema is known to occur in an infarction, a trauma or status epilepticus [15]. Cytotoxic edema is caused by a decrease in intracellular ATP and a failure of the sodium-potassium pump. This will cause an increase in the osmotic gradient and the transport of water into cells, resulting in cellular swelling [15]. Cytotoxic edema shows hyperintensity on diffusion-weighted (DW) images associated with decreased ADC values. In addition, tumors, hemorrhages, abscesses and coagulative necrosis also result from a decrease in ADC values [15]. Histopathologic characteristics of PM include hemorrhage and necrosis [18]. In the PM, we assumed that cytotoxic edema and/or hemorrhage would occur in the whole spinal cord. The hyperintense swollen region in T2WI seemed to be due to cytotoxic edema of neuronal fiber bundles. This hypothesis was able to be induced from the results of the examination of DTI.

In the meningioma case in this study, the patient underwent hemimaminectomy and the tumor was removed surgically, but tumor recurrence was confirmed by MRI examination after two months from surgery. The removed tumor was diagnosed as meningioma by histopathological examination. The first MRI/DTI examination was performed prior to the administration of an anticancer agent when the tumor recurrence was detected. The second MRI/DTI examination was performed at six months after initial chemotherapy. In the post-chemotherapy examination, no tumor shrinkage was observed with conventional MRI. However, the patient’s ability to stand and walk was improved after six months of receiving the anticancer agent (data not shown). After anticancer chemotherapy, the average ADC value of lesion site increased, and the average FA value of lesion site decreased. These were supposed that the pressure to the spinal cord slightly was reduced due to the property change of the tumor and the suppression of tumor growth by the administration of anticancer drugs. At compression sites without spinal cord injuries (edema and demyelination), the ADC values decrease, and the FA values increase [6]. Therefore, it was assumed that the pressure by the tumor was high before anticancer chemotherapy. After anticancer chemotherapy, the ADC value was higher, and the FA value was lower at lesion sites than each value at normal sites. Generally, higher ADC value and lower FA value mean axonal vasogenic edema [6]. However, this dog could stand and walk after anticancer chemotherapy. Even if it has higher ADC value and lower FA value, we considered that spinal cord of this dog has a function near normally. We could not evaluate influence of the injury by the removed surgery, but the release from tumor and vertebra cavity pressure might result in higher ADC value and lower FA value. Fiber tractography in meningioma case indicated an improvement in the continuity of fiber trajectories from the cranial side to caudal side through the tumor. In cerebral tumors cases of human, it is reported that tract alteration patterns of white matter tract, such as deviation, deformation and apparent tract interruption, improved after the operation [12, 24]. Based on results shown, it was hypothesized that the continuity of the nerve fiber had initially been inhibited by the pressure of a growing tumor and that this was improved after six months when the pressure subsided.

Since EPI-based DW imaging typically features a low signal noise ratio (SNR), it is important to obtain accurate ADC, FA and fiber-trajectory-imaging datasets in reasonable timeframes [2]. This timeframe problem is especially crucial in veterinary clinical MRI scans, because the anesthetization of animals is necessary. Skare et al. reported that it was necessary to increase the number of non-collinear directions DW gradient (motion probing gradient; MPG) schemes, as this greatly reduces the standard deviation of the FA and its dependence on the orientation of the diffusion ellipsoid [20]. Using many diffusion-weighting gradient schemes, such as 42, 162 or 642 directions, would provide higher SNR and more accurate diffusion ellipsoids [13]. However, this would also result in long sequence times, which may be unsuitable in veterinary clinical MRI scans [2]. For comparison, human clinical DTI is performed in MPG schemes of 6 or 12 directions [23], whereas animal experiments are performed in 30 or 42 directions [2, 3]. In our pilot studies using 12- and 30-direction MPG schemes with calf cervical and lumbar spinal cords, the fiber trajectories of the neuronal fiber were well represented. Asanuma et al. reported that a 30-direction MPG scheme was suitable in order to obtain optimized fiber trajectories in tumor-bearing rat brain DTIs [2]. Two reasons are thought to explain this: brain nerve fibers are oriented in various directions, and the size of a rat brain is very small. To detect water diffusions in small-sized brains with complicated neuronal directions—such as rat brains—30-direction MPG is considered to be suitable for nerve fiber tractography. However, our pilot study indicated there was no difference in the fiber trajectory using 12- and 30-direction MPG schemes in calf cervical and lumbar spinal cords. Most spinal cords were seen to be unidirectional, i.e., running in the cranial-caudal direction. When the limitations of anesthesia time were considered, we admitted that fiber tractography of spinal cords could adequately visualize a subject using 12-direction MPG schemes in veterinary clinical MRI scans.

In conclusion, in order to evaluate the functional continuity of the spinal cord in various spinal cord disease cases in veterinary medicine, we imaged fiber tractography of spinal cords and compared their diffusion parameters, including the average ADC and FA values, using clinical MRI scans. Fiber tractography visualized spinal cord injury as the discontinuity of the spinal cord, such as rupture of fiber trajectories, loss of neuronal bundles and disorder of fiber directions. In thoracolumbar IVDH cases, fiber tractography indicated that the discontinuity of fiber trajectories was closely associated with neurologic grade. In the PM case, the ADC and FA values of the hyperintense swollen region in T2WI seemed to decrease due to cytotoxic edema and hemorrhage in the whole spinal cord. In the meningioma case, fiber tractography indicated the recovery of spinal cord continuity through the administration of an anticancer agent. Further studies in more cases are needed, because PM and meningioma cases had few cases. These results of this study suggest that fiber tractography and DTI may have the potential to aid in the diagnosis and prognosis of spinal cord diseases in veterinary clinical medicine.
REFERENCES


doi: 10.1292/jvms.16-0504