Acupuncture effects on dogs with Intervertebral Disk Disease: A Pilot Study

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Research Article

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Abstract

Sixteen dogs with intervertebral disk disease (IVDD)(3–16 years old) were examined acupuncture effects of energy metabolism changes. Acupuncture treatments were performed at acupuncture points according to individual needs to the dogs. Acupuncture effects were evaluated based on the IVDD evaluation scales. Pyruvate and lactate concentrations, LDH and MDH activities, malate dehydrogenase/lactate dehydrogenase ratio (M/L ratio), and LDH isozyme electrophoretic patterns were measured as energy metabolism markers after the acupuncture treatments. In dogs with IVDD improved group, plasma pyruvate concentrations, LDH and MDH activities were significantly decreased, and M/L ratio were significantly increased. Plasma LDH isozyme electrophoretic patterns changed from LDH5 predominance to LDH1 predominance. The present study suggests that local redox potential is improved and energy metabolisms are activated in dogs with IVDD after the acupuncture treatments. Acupuncture treatments on dogs with IVDD may result in activation of citric acid cycle and increase in ATP production followed by improvement of IVDD. Further studies with a large number of dogs will be needed to clarify this hypothesis.

Introduction

Intervertebral disk disease (IVDD) is considered to be one of causes to induce gait disturbances in dogs (Jeffery et al. 2013). Canine IVDD can be very common within chondrodystrophic (CD) breeds such as Miniature Dachshund, French Bulldog, Welsh Corgi, Toy Poodle, Pekingese, and Shih Tzu. These CD breeds dogs suffer from herniated discs much earlier than non-chondrodystrophic (NCD) breeds. Recently, the prevalence of canine IVDD has increased with age. (Smolders et al. 2013). Although acupuncture treatment for canine IVDD has recently increased (Han et al. 2012, Liu et al. 2016, Roynard et al. 2018, Wright 2019), biochemically analyzed reports for acupuncture effects are very few. We have shown that plasma M/L ratio is one of the potential useful indicators of oxidative metabolism and increased M/L ratio suggests increased ATP production via citric acid cycle (Li et al. 2012). In this pilot study, we measured plasma M/L ratio, pyruvate and lactate concentrations, LDH and MDH activities, and LDH isozyme electrophoretic patterns after acupuncture treatments. Although this is a pilot study, we found the acupuncture effects regarding energy metabolism on dogs with IVDD.

Materials And Methods

Animals

We investigated acupuncture effects on 16 dogs with IVDD. Canine age ranged 3–16 years old. Breeds included Miniature Dachshund (8), Norfolk Terrier (2), Chihuahua (1), Miniature Schnauzer (1), French Bulldog (1), Maltese (1), Jack Russell Terrier (1), and Mixed (1). The dogs were divided into two groups by acupuncture effects (Table 1). Before treatments, authors gave written informed consents to all dog's owners. Ethical approval for this study was obtained from Higashi Chiba Animal Medical Center (Togane
City, Chiba) (R2-1). Authors confirm this study was conducted following best veterinary practice procedures.

**Acupuncture**

Acupuncture treatment method applied in this study was based upon the traditional Chinese veterinary medicine and Japanese methods (Sawamura 2019). Acupuncture needles used in this study were as follows: 1) Seirin D-Type No.1. (φ0.16) x 15 mm, 2) D-Type No.3 (φ0.20) x 15 mm, 3) Kanaken Disposable Acupuncture φ0.16 x 30 mm, φ0.18 x 30 mm, and 4) Kanaken Disposable Acupuncture φ0.20 × 30 mm needles were applied.

Acupoints were GV14, GV20-01, BL18, BL23, BL26, BL28, BL40, ST36 (Sawamura 2019). Electric acupuncture was performed for 20 minutes using Lasper Ace manufactured by Kanaken Co., Ltd. Acupuncture needles are placed into acupuncture points and left for 15 minutes, whereas electric currents are passed along the needle as a form of low frequency pulse therapy. Acupuncture effects were evaluated as a grade improvement in the IVDD grading scale (Oji 2015). In classification of cervical spinal cord disorders, grade 1 has with neck pain without neurological abnormalities; grade 2 also has gait disturbances and neurological abnormalities; grade 3 also has difficulty standing and walking and neurological abnormalities. In classification of thoracolumbar spinal cord disorders, grade1 has no spinal cord dysfunction but back pain; grade 2 has paresis of hind limbs; grade 3 has severe paresis of hind limbs; grade 4 has hindlimb paralysis and deep pain sensation; grade 5 has paralysis of the hind limbs and no deep pain sensation (Oji 2015). Acupuncture effect has been evaluated for 2 months after treatment except sample No.14 and 15.

**Blood sampling and assays of metabolites and enzymes**

Blood (2.5 mL) was collected from jugular veins with heparin as anti-coagulant before and after acupuncture treatment, both within approximately 30 minutes. After centrifugation at 1,200rpm for 5 minutes at 4°C, plasma was obtained and stored at -40°C until use for assay.

**Measurements of Plasma metabolites concentrations and enzyme activities**

Plasma pyruvate concentrations were measured by a previously described method (Czok and Lamprecht 1974) and lactate concentrations were measured with commercial kit (Lactate Assay Kit-WST, Dojindo, Tokyo, Japan). Plasma lactate dehydrogenase (LDH) (Kaloustian et al. 1969) and malate dehydrogenase (MDH) (Bergmeyer and Bernet 1974) activities were measured by previously described methods, respectively. The plasma MDH/LDH (M/L) ratio was calculated as MDH activities divided by LDH activities. Plasma LDH isozyme patterns were detected by the biphasic agarose gel electrophoresis method utilizing commercial Quickgel LD gels (Helena Laboratories, Saitama, Japan) (Hirakawa et al.2012). LDH fraction were assessed and analyzed using Quick Scan (Helena Laboratories, Saitama, Japan).

**Statistical Analysis**
Results are presented as means ± standard deviation (SD). Statistical significance was determined by paired-T test. The significant level was set at P < 0.05.

Results

As shown in Tables 1, dogs with CVDH, G2 and G3 and IVDD G2,3,5 showed improved grades. The dogs without improvement included IVDD, G2 and G3,5 with other disorders. As shown in Tables 2, in dogs with improved IVDD group, plasma pyruvate concentrations were significantly decreased after acupuncture treatment (0.17 ± 0.08 mM to 0.10 ± 0.03 mM). In addition, plasma LDH activities decreased from 67.06 ± 24.81 IU L⁻¹ to 58.65 ± 22.10 IU L⁻¹, while MDH activities increased from 55.69 ± 37.76 IU L⁻¹ to 64.94 ± 42.56 IU L⁻¹. M/L ratio was significantly increased from 1.24 ± 1.19 to 2.80 ± 3.78 after acupuncture treatment. Plasma lactate concentrations did not change significantly. In the dogs without improvement group, plasma pyruvate and lactate concentrations and LDH and MDH activities were not changed significantly after acupuncture treatment.

Representative plasma LDH isozyme electrophoretic patterns in dogs with IVDD were showed in Fig. 1. After acupuncture treatment, LDH 5 fraction decreased from 65.07–38.83% while LDH1 fraction increased from 17.40–31.84% in 10 dogs of IVDD improved group (Table 2). Six dogs in non-improvement group were not changed.

Discussion

Intervertebral disk disease (IVDD) is often observed in dogs and its prevalence has increased with aging. Recently, Chinese traditional medicine has been applied to IVDD treatments (Han et al. 2012, Liu et al. 2016, Roynard et al. 2018, Wright 2019). It has been reported that acupuncture treatments was effective in alleviating oxidative stress in various diseases (Watanabe and Shinohara 2008, Su et al. 2020, Zen et al. 2014). In this study, we measured plasma metabolites concentrations and enzymes activities, which relates to energy metabolism in dogs with IVDD after acupuncture treatment. LDH5 fraction in canine plasma is generally predominant (Washizu et al. 2002). After acupuncture treatments, dogs of IVDD improved group decreased plasma pyruvate concentrations and increased LDH1 fractions. This suggests that the acupuncture treatments may change local redox potential (Watanabe and Shinohara, 2008, Zeng et al. 2014), and increase NAD to lead the flow from lactate to pyruvate and activate citric acid cycle. LDH 1, which shows a lower Km value for pyruvate, usually function under aerobic condition (Nonaka et al. 1989). Plasma M/L ratio is one of the potential useful indicators of oxidative metabolism. Increased M/L ratio may suggest increased ATP production via citric acid cycle (Li et al. 2012). Limitations in this study are as follows.

1. This study is a pilot study. Studies with a large number of dogs should be made to confirm these findings.
2. Long term follow-up study after the acupuncture treatments could not be performed due to lack of pet owner’s consensus although acupuncture effect may be transient.
3. Acupuncture points were difficult to confirm. In this study, acupuncture treatments were performed by an experienced person using certain acupoints in a similar procedure.

### Abbreviations

- **ATP**  adenosine triphosphate
- **CD**  chondrodystrophic
- **IVDD**  intervertebral disk disease
- **LDH**  lactate dehydrogenase
- **MDH**  malate dehydrogenase
- **M/L ratio**  malate dehydrogenase/lactate dehydrogenase ratio

### Declarations

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#### Author contribution

The study was designed by Koh Kawasumi. Data collection and analysis were performed by Megumi Sawamura and Toshiro Arai. All authors read and approved the final manuscript.

#### Funding

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#### Data availability

The datasets generated during present study are available from the corresponding author on request.

#### Ethics approval

The study was approved by the Ethic Committee of Higashi Chiba Animal Medical Center (Togane City, Chiba) (R2-1).

#### Consent to participate
All authors consent to participate in this publication.

Consent for publication

All authors consent to publish the manuscript.

Conflicts of interests

All authors declare that they have no conflict of interest.

References


Tables

Table 1-2 are available in the Supplementary Files section.

Figures
Figure 1

Representative LDH isozyme electrophoretic patterns in plasma of a dog with IVDD before (A) and after (B) the acupuncture treatment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.docx