Canine X-Linked Muscular Dystrophy in Japan (CXMD_j)

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Abstract: The purpose of this study was to develop a strain of canine X-linked muscular dystrophy (CXMD), a model of Duchenne muscular dystrophy, in Japan. A female beagle was artificially inseminated with frozen-thawed spermatozoa derived from an affected golden retriever. Subsequently, two carrier female dogs (G1 carriers) and four normal male littermates were produced. Thereafter, the two G1 carriers were mated with beagle sires. As a result, each bitch whelped three times, and out of 54 pups, 17 affected male descendants, and 11 carrier female descendants (G2 carriers) were detected. One G2 carrier was then mated with a beagle sire and 15 pups in two whelpings were produced, including five affected males and four carrier females (G3 carriers). A total of 10 female beagles were artificially inseminated to evaluate the fertility of the frozen-thawed spermatozoa from the two affected dogs. The whelping rates of the two affected dogs were 4/5 and the litter sizes were 5.0 ± 1.41 and 6.0 ± 0.82, respectively. These results indicate that a canine X-linked muscular dystrophy colony has been established in Japan. We called them CXMD_j.

Key words: canine, X-linked, muscular dystrophy, frozen-thawed, spermatozoa

Introduction

Muscular dystrophies in humans are a heterogeneous group of inherited, progressive, degenerative myopathies characterized morphologically by muscle-fiber hypercontraction, necrosis, and regeneration [11]. The most common and the most devastating of the dystrophies is X-linked Duchenne muscular dystrophy (DMD), which is caused by mutations in the dystrophin gene [12]. Canine X-linked muscular dystrophy (CXMD) which has a splice site mutation in the dystrophin gene has been recognized and best characterized in the golden retriever breed in which it occurs spontaneously. Moreover, the signs and symptoms of canine disorder are

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similar to those of human DMD [8, 16].

Dystrophin deficiency has been reported in two other animal models of DMD: the X-linked muscular dystrophy mouse [2], and the hypertrophic feline muscular dystrophy cat [3, 6]. These two models do not show significant muscle weakness, so CXMD is expected to be the best model for human DMD [18].

The purpose of this study was to develop a strain of canine X-linked muscular dystrophy by 1) artificial insemination (AI) with frozen-thawed spermatozoa derived from an affected golden retriever in a beagle bitch, and by 2) the interbreed crossing of carrier female dogs derived from an affected golden retriever with beagle sires. The canine model smaller than the golden retriever, has an appropriate body size for muscular dystrophy and is more easily handled by a person. The clinical signs are mild [17], therefore, the model dog may live a long time, and experimental designs must incorporate this factor.

Materials and Methods

Animals

Thirteen 2- to 6-year-old multiparous and three 1- to 8-year-old male CSK Beagles (Chugai Research Institute for Medical Science, Inc., Nagano) were used in this experiment. The frozen spermatozoa derived from a golden retriever affected with X-linked muscular dystrophy were used to produce a first generation of carrier female dogs. These beagles and carriers were individually housed in stainless steel cages (142 × 83 × 115 cm) with ad libitum access to fresh water. They were also given a pelleted diet (CD-5M, Clea Japan Inc., Tokyo) of 300 g a day. Room temperature was maintained at 20 to 25°C and relative humidity was 40 to 70%. All animals in this experiment were treated in accordance with the guidelines provided by the Ethics Committee for Treatment of Laboratory Animals of Chugai Pharmaceutical Co., Ltd. (Tokyo).

Detection of luteinizing hormone surge

Blood samples were collected from the venae cephalica of the antebrachium of female dogs after the onset of proestrus. Serum was separated by a routine method, and immediately after the separation, the Canine Ovulation Timing Test (ICG Status-LH™, Synbiotics Co., USA) was applied to detect the luteinizing hormone (LH) surge.

Artificial insemination using frozen-thawed spermatozoa derived from an affected golden retriever and mating of the produced carrier female dogs with beagle sires

At 4, 5 and 6 days after the LH surge, a multiparous beagle was artificially inseminated with the frozen-thawed spermatozoa obtained from an affected golden retriever. The frozen spermatozoa were obtained from the colony with golden retriever muscular dystrophy at the University of Missouri-Columbia. The straws containing 200 × 10⁶ spermatozoa were thawed in a water-bath at 75°C for 12 sec and emptied into a tube. The thawed spermatozoa were then diluted 1:1 with egg-yolk Tris extender at 37°C, then deposited on the cranial vagina using a miniature pig semen injector [14]. The produced carrier females were mated with male beagles at 3 and 6 days after their LH surge.

Genotyping of CXMD allele

A snapback method of single-strand conformation polymorphism analysis [7] was used to determine the CXMD allele.

Measurement of body weights and examination of clinical signs

The measurement of body weights and the examination of clinical signs of affected dogs, carrier female dogs and normal littermates were carried out daily for 4 weeks after birth, and then once every 2 weeks.

Stock of frozen spermatozoa collected from X-linked muscular dystrophy dogs and evaluation of its fertilizing ability after artificial insemination

The method used to freeze spermatozoa from the two affected dogs was the same as that described by Shimatsu et al. [14]. The first and second (sperm-rich) fraction of each ejaculate was collected by digital manipulation. Thereafter, a total of 10 multiparous beagles were artificially inseminated to evaluate the fertility of the frozen-thawed spermatozoa at 4, 5 and 6 days after the LH surge.

Data analysis

The data of growth rate and litter size were subjected to a one-way ANOVA test. All calculations were performed using StatView software (SAS Institute Inc., USA).
Results

A female beagle artificially inseminated with frozen-thawed spermatozoa derived from an affected golden retriever whelped two carrier female dogs (G1 carriers) and four normal male littermates (Fig. 1). Each G1 carrier mated with male beagles whelped three times, and out of 54 pups, 17 affected male descendants, and 11 carrier female descendants (G2 carriers) were detected. Fifteen pups in two whelpings were then produced from one G2 carrier, including five affected males and four carrier females (G3 carriers) (Fig. 1).

Comparative growth rates of pups derived from spermatozoa of an affected golden retriever, of affected male pups of G1 and G2 carriers, and of other pups of G1 and G2 parents are shown in Fig. 2. No significant difference was detected between these three growth rates; however, the data of affected male pups born from G1 and G2 carriers crossed in the narrowest curve.

Suckling by most affected pups was weak during the lactation period so their body weights decreased accordingly; therefore, vicarious post-partum beagles were used as surrogate mothers. Indeed, 3- to 4-month-old affected dogs could not fully open their jaws, drooled continuously, and moved their hind limbs simultaneously. Five out of 22 affected dogs died between 5 to 9 months of age.

The fertilizing ability of frozen-thawed spermatozoa from the two affected dogs was examined using AI (Table 1). Four out of five bitches inseminated with these two dogs whelped, and the litter sizes were 5.0 ± 1.41 and 6.0 ± 0.82, respectively.

Discussion

Seventeen carrier female dogs and 22 affected male dogs were produced (Fig. 1). Specifically, two first generation carriers were born by AI with frozen-thawed spermatozoa. We reported previously that canine AI with frozen-thawed spermatozoa should be carried out at 4, 5 and 6 days after the bitch’s LH surge [14]. In this study, the same AI system was used, resulting in six pups, which was similar to the accepted litter size for the CSK Beagle breed by natural mating (5.3 ± 2.37, unpublished data). The litter size of G1 and G2 carrier mothers ranged from 6 to 10 (Fig. 1). Golden retrievers whelped an average of 8.1 pups [4] and the reproductive ability of golden retriever-beagle crosses may not decrease.

Only one out of 22 affected pups died shortly after birth (due to asphyxia) (Fig. 1). Valentine et al. [17] examined clinical signs of affected dogs and found that some affected pups died within the first 10 days after birth. The suckling action of most affected pups was weak during the lactation period, resulting in body-weight loss. Therefore vicarious post-partum beagles were used in our study so that pups could suckle easily.

The average body weight of affected dogs was 11.5 kg at 12 months of age (Fig. 2), which was slightly heavier than the accepted weight of adult CSK Beagle males at the same age (10.9 ± 1.04 kg, unpublished data). The beagles were appropriate body sizes, had good temperaments, and were easily handled by all persons [1]. Affected golden retriever-beagle crosses can be good models for DMD at least in this aspect.

It was noticed that, 3- to 4-month-old, affected dogs showed a fatigability, an inability to fully open their jaws, drooled continuously, and moved their hind limbs simultaneously. Indeed, early muscle atrophy was evident and was most prominent in their truncal and head temporal musculature. Moreover, there was progressive enlargement at the base of the tongue so it was weak. Thus these dogs had difficulty masticating their food. Similar clinical signs in affected dogs have been reported in other studies [5, 16, 17]. Five out of 22 affected dogs died between 5 to 9 months of age in our study. The immediate causes of these dogs’ death are not yet clear.
The frozen-thawed spermatozoa from the two affected dogs were shown to have high fertilizing ability (Table 1). Whelping rates and litter sizes did not differ compared with our previous study using frozen-thawed spermatozoa collected from normal beagles [14], and whelping rates were similar to the values obtained in numerous studies [9, 10, 13–15]. The fertilizing success of frozen spermatozoa obtained from affected dogs is deemed important so that a strain of CXMD can be maintained.

The results of the present study suggest that a colony of canine X-linked muscular dystrophy has been established in Japan. We called our dystrophic dogs CXMDJ.

Acknowledgments

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Table 1. Fertilizing ability of frozen-thawed spermatozoa from two dogs affected with X-linked muscular dystrophy

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>No. of beagles whelped/inseminated</th>
<th>Litter size</th>
</tr>
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<tbody>
<tr>
<td>C04</td>
<td>4/5</td>
<td>5.0 ± 1.41</td>
</tr>
<tr>
<td>C12</td>
<td>4/5</td>
<td>6.0 ± 0.82</td>
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</tbody>
</table>

Values are mean ± SD.

References


