Experimental Chemonucleolysis with Chymopapain in Canine Intervertebral Disks

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ABSTRACT. The present study describes the radiological and histological changes in the canine intervertebral disk after the experimental injection of chymopapain as the chemical reagent, and determines the appropriate dose of the enzyme for treatment of herniated disks. By radiography, narrowing of the disk space was observed within 2 weeks after the injection of chymopapain, and recovered to 74.1% in the 0.1 mg group, 61.1% in the 1.0 mg group and 71.7% in the 10.0 mg group at 12 weeks. The disk space recovery showed a tendency to delay with aging. Microscopically, proteoglycan positive matrix appeared and the nuclear space was reduced in each disk at 2 weeks after chymopapain injection. The nucleus pulposus contained an irregularly-defined mass consisting of clusters of degenerated notochordal cells surrounded by proliferated chondrocytes and collagen matrix. In each disk at 12 weeks after chymopapain injection, the center of the nucleus pulposus was replaced by fibrocartilage tissue. In the disk into which 10.0 mg chymopapain was injected, the nuclear space filled with dense fibrocartilage tissue without a regenerated matrix component and narrowing of the disk were maintained. It is suggested that canine chemonucleolysis with 10.0 mg of chymopapain reduces the interdiskal pressure. This treatment may therefore relieve the signs and symptoms of herniation of the nucleus pulposus, and may effect chemical disk decompression.—**KEY WORDS:** canine intervertebral disk, chemonucleolysis, chymopapain.

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Chemonucleolysis with proteinases to reduce interdiskal pressure was first suggested as a possible treatment for human low-back pain and sciatica in 1959 [11]. The first clinical use of chymopapain was reported in 1964 [14]. Since then, chemonucleolysis together with chymopapain has been widely used in Canada and Europe as an alternative treatment for human herniated disk. After the therapeutic compound was approved in 1983 by the U.S. FDA, chemonucleolysis for chemical decompression became the most effective treatment for human herniated disk in North America and Europe [16].

In the veterinary field, Saunders [13] reported in 1964 that chemonucleolysis was a promising therapy for canine herniated disk. In countries where therapeutic chymopapain has government approbation, canine chemonucleolysis was reported [1, 8] to be the choice for this disease without improvement of the signs and symptoms by conservative treatments with anti-inflammatory drugs, orthopedic appliances or thermotherapy [4–6]. The usefulness of experimental therapeutic trial with chymopapain as the chemical reagent is reported in human medicine [2, 3]. However, in veterinary medicine, the experimental and therapeutic evaluation of chemonucleolysis including the optimal dose of the reagent has rarely been confirmed. The purpose of the present study is to examine radiologically and histologically the effective dose for the application of chemonucleolysis with chymopapain as the chemical reagent.

10.0 kg were used in this study. Before the needle insertion into the lumbar intervertebral disks, the animals were anesthetized with intramuscular injections of xylazine hydrochloride (2 mg/kg) and ketamine hydrochloride (15 mg/kg).

Chemonucleolysis technique: Chymopapain (5.0 units/mg, SIGMA Chem. Co., U.S.A.) as the chemical reagent was dissolved at 3 graded levels (1.0 mg/ml, 10.0 mg/ml

Fig. 1. Needle placement confirmed by x-rays.

Fig. 2. Points in intervertebral space measured to calculate the disk height (HI).

MATERIALS AND METHODS

Animals: Five clinically normal mongrel dogs with the age from 8 months to 3 years old and weighing from 8.0 to

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and 100.0 mg/ml) in physiological saline and sterilized through a 0.22 μ m filter (Millex-GS, Nihon-Millipore Co., Japan). After anesthesia, the animals were placed on their dorsoventral side on an X-ray table. The skin was surgically prepared and 21-gauge $\times$ 1.5-inch (0.80 $\times$ 38 mm) disposable needles were inserted into the lumbar intervertebral disks among L1-L5 vertebrae by the 45-degree-angle dorsolateral approach [13]. The needle placement was determined by viewing the shadow on the image intensifier screen (Fig. 1). The inserted needle was fixed with a Luer-Lok tuberculin glass syringe. Chymopapain, at doses of 0.1 mg (Disk-1), 1.0 mg (Disk-2) and 10.0 mg (Disk-3) in 0.1 ml of physiological solution, or 0.1 ml of control physiological solution (Disk-C), was injected into the nucleus pulposus of each disk.

Radiological examinations: Radiography of the disks in three dogs (8 months, 16 months and 3 years old) was undertaken at 1, 2, 4, 8 and 12 weeks after the injection. The intervertebral spaces were measured and the disk height index (HI) [8] was calculated from Fig. 2 as follows:

$$HI = \frac{ac+bd}{2ab} \times 100$$

where ab was the width of the vertebrae, and ac and bd were the height of intervertebral spaces. The mean HI was calculated for the three dogs.

Histological examinations: The two other dogs (14 months old) were killed with an intravenous injection of an overdose of pentobarbital sodium at 2 and 12 weeks after the chymopapain injection. The vertebrae and intervertebral disks were harvested and placed in neutral-buffered 10% formalin. Each bone was decalcified in a formalin-formic acid solution (10% formalin solution: formic acid, 9:1) and was then split along its long axis. The inner surface of one half was embedded in paraffin and

sectioned at 5 μ m. The resulting sections were stained with hematoxylin and eosin, safranin-O, toluidine blue, and Azan.

RESULTS

Radiological findings: The mean HI of the intervertebral space as seen by radiography in the three dogs is shown in Fig. 3. The mean HI of Disk-C before saline injection was 16.9, and ranged from 16.0 to 17.2 after the injection. Narrowing of the nucleus pulposus after the chymopapain injection were seen at about 1 and 2 weeks. The mean HI of Disk-1 was 17.0 before the injection, but the HI had decreased to 6.4 at 1 week after the injection and then increased gradually to 12.6 at 12 weeks. Mean HI of Disk-2 was 17.5 before the injection, against 6.8 at 2

Fig. 3. The mean HI of intervertebral space in radiographs of three dogs. Chymopapain 0.1 mg (■), 1.0 mg (▲) and 10.0 mg (●) and physiological saline (◇) were injected into the vertebral disks.

Fig. 4. At 2 weeks after chymopapain injection, longitudinal section through intervertebral disks between L1 and L5 vertebrae showing the effects of the enzyme. Top: Longitudinal section through a decalcified lumbar spine, vertebral bodies and intervertebral disks injected with chymopapain (left to right: 10.0 mg, 1.0 mg, saline control and 0.1 mg). Bottom: Toluidine blue staining sections of the 4 lumbar intervertebral disks (left to right: saline control, 0.1 mg, 1.0 mg and 10.0 mg). In saline control disk, the nucleus pulposus was dense and bulging, and filled the inner disk space.

Fig. 5. At 12 weeks after chymopapain injection, longitudinal section through intervertebral disks between L1 and L5 vertebrae showing the effects of the enzyme. Top: Longitudinal section through a decalcified lumbar spine, vertebral bodies, and intervertebral disks injected with chymopapain (left to right: 1.0 mg, saline control, 10.0 mg and 0.1 mg). Bottom: Toluidine blue staining sections of the 4 lumbar intervertebral disks (left to right: saline control, 0.1 mg, 1.0 mg and 10.0 mg). The inner space of 0.1 mg and 1.0 mg disks recovered to the saline control but in the 10.0 mg was narrowing.

weeks after and then increased gradually to 10.7 at 12 weeks after the injection. The mean HI of Disk-3 was 19.1 before the injection, fell to 8.1 at 1 week after and then increased gradually to 13.7 at 12 weeks after the injection. The disk space narrowing after the injection of chymopapain recovered to 74.1% in the 0.1 mg group, 61.1% in the 1.0 mg group and 71.7% in the 10.0 mg group within 12 weeks, respectively. At 12 weeks, the 0.1 mg and 1.0 mg injected disk spaces in an 8-month-old dog recovered to the pretreatment level but the disk space injected with 10.0 mg chymopapain narrowed.

Gross findings: At 2 weeks after chymopapain injection, the gross effects of the enzyme on nucleus pulposus were seen in longitudinal sections of lumbar vertebrae (Fig. 4). Nucleus pulposus of Disk-C was dense and bulging, and filled the inner disk space, whereas the inner disk space and nucleus pulposus in chymopapain-injected Disk-1, -2 and -3 were narrowing. At 12 weeks after chymopapain injection recovery of the narrowed nucleus pulposus was seen in longitudinal sections of lumbar vertebrae (Fig. 5). The Disk-1 and Disk-2 spaces recovered to the level of Disk-C, but Disk-3 narrowed. The Disk-1 and Disk-2 nucleus pulposus recovered nearly to the dense and bulging level of Disk-C, but in Disk-3 there was narrowing of the nucleus pulposus. The nucleus pulposus of Disk-1 and -2 filled the inner disk space as well as Disk-C, but that of Disk-3 was narrowing.

Histological findings: Narrowing of the nucleus pulposus in the chymopapain injected disk correlated well with the gross findings mentioned above. In the control disk (Disk-C) examined at 2 weeks after saline injection, the matrix containing proteoglycan as indicated by remaining safranin-O staining, and notochordal cells were scattered in the matrix. On the other hand, most of the proteo-

Fig. 6. Microscopic findings of the nucleolus pulposus in the disk obtained at 2 weeks after chymopapain injection (10 mg injected disk). Decrease and condensing of the nuclear components of the nucleus pulposus with narrowing of disk space are observed. Clusters of the degenerated notochordal cells are scattered in the homogeneous matrix of the nucleus pulposus. H-E stain, $\times 45$.

glycan positive matrix disappeared and nuclear spaces were reduced in each chymopapain injected disk. The

Fig. 7. Microscopic findings of the nucleus in the disk obtained at 12 weeks after chymopapain injection (10.0 mg injected disk). Zonal structure of proliferated cartilage tissues connected to bony endplate of the vertebral bone is evident. Fibrocartilage tissue (*) of the nucleus pulposus is also seen. AZAN stain, $\times 110$.

Fig. 8. Microscopic finding of the nucleus pulposus in the disk obtained at 12 weeks after chymopapain injection (10.0 mg injected disk). Nucleus pulposus is completely replaced by fibrocartilage tissues. Toluidine blue stain, $\times 110$.

nucleus pulposus contained an irregularly-defined mass consisting of a cluster of degenerated notochordal cells surrounded by proliferated chondrocytes and collagen matrix (Fig. 6). These findings were more apparent in Disk-3 (10 mg injected disk).

In each disk examined at 12 weeks after chymopapain injection, the center of the nucleus pulposus was replaced by fibrocartilage tissues. Furthermore, the proliferated cartilage zone connected to the bony endplate of the vertebral disk was characteristic (Fig. 7). However, in Disk-1 (0.1 mg injected disk) and Disk-2 (1.0mg injected disk), homogeneous matrix containing proteoglycan was also present between fibrocartilage tissues and the peripheral cartilage zone. These changes resulted in

bulging of the nucleus pulposus similar to that in the control disk at 12 weeks after saline injection. In Disk-3, the nuclear space filled with dense fibrocartilage tissues without both regenerated matrix component and narrowing of the disk was retained (Fig. 8).

DISCUSSION

Chymopapain derived from papaya latex is a proteolytic enzyme [7], and has the ability to disrupt the nucleus pulposus of the intervertebral disk [14]. The enzymatic dissolution of proteoglycan in a nucleus pulposus lesion changes the intradiskal pressure and relieves a localized pathogenic condition such as a herniated intervertebral

disk in man [15] and dog [1, 13].

It is reported that gross change in the nucleus pulposus of rabbits following the injection of effective dose levels was usually first seen at one week and reached a maximum at two weeks [7]. The canine disk height within 2 weeks after chymopapain injection decreased to less than one-third the normal height [2]. Our radiographs of the canine lumbar intervertebral disk showed maximum dissolution of the nucleus pulposus within 2 weeks after chymopapain direct injection, but there were no complications such as apparent effects on the surrounding tissues, for example ligaments, bone, nerve roots, and dura mater. The enzymatic dissolution of the nucleus pulposus decreased the intradiskal pressure, and the loss of metachromatic staining decreased the proteoglycan of the nucleus pulposus. Gravin and Jinnings [8] reported that the disk height is directly related to the dose of chymopapain and the age of the dogs. Hayashi [10] showed that the nucleus pulposus and the inner layer of the anulus fibrosus were digested in rabbits undergoing high dose injection. At a lower dose the anulus fibrosus remained intact and the posterior inner layer of the anulus fibrosus mainly proliferated toward the anterior portion of the disk, filling the space around the digested nucleus pulposus, and restoring disk height. The canine nucleus pulposus regenerated after the chymopapain chemonucleolysis, and the tissues recovered to normal levels within about 3 months [2]. Our results showed that the mean disk height recovered to 61.1% (Disk-2) and 74.1% (Disk-3) of the preoperative level within 12 weeks after chymopapain injection, but the mean disk height of a dog aged 8 months injected with 0.1 mg and 1.0 mg recovered to the preoperative level. Human chondroid and fibrosus of the nucleus pulposus changes with age [15]. Young rabbits recover disk height more rapidly than mature ones [10]. Our results suggested a tendency to a delay in the disk space recovery with age. Further investigation may be needed to clarify the age related change in disk space restoration in dogs.

The histological changes observed in the present experiments were essentially the same as those described by other investigators, although the number and age of the experimental dogs were limited [2, 8]. However, proliferation of cartilage tissues connected to the bony endplate of the disk was characteristic of the disks examined at 12 weeks after chymopapain injection. This change was thought to be a peculiar response of a chymopapain injected disk, because the change was not observed in Disk-C. On the other hand, regeneration of proteoglycan had been suppressed by a 10 mg dose of chymopapain at 12 weeks after its injection. Bradford *et al.* [3] reported that reconstitution of the disk space with resynthesis of proteoglycan can occur with time in a chymopapain injected disk. Therefore, the long-term effective dose of chymopapain injected may depend on the degree of regeneration of nuclear contents such as proteoglycan.

It is known that the nucleus pulposus undergoes either chondroid or fibrous change with age [9]. Lumbar and

cervical chemonucleolysis was evaluated in dogs aged 3.4 years and older by Atilola *et al.* [1]. They found them very useful for the treatment of Hansen's type II protrusion [9], in which the nucleus is contained and only a partial bulge of the dorsal anulus fibrosus is seen.

In conclusion, we consider that canine chemonucleolysis with 10 mg of chymopapain reduces interdiskal pressure. Therefore, this treatment may relieve the signs and symptoms caused by protrusion of the nucleus pulposus from the intervertebral disk, and may effect chemical disk decompression.

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