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Intradiscal pressure after intradiscal injection of hypertonic saline: an experimental study

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Abstract Although chemonucleolysis with chymopapain is a long-established treatment for lumbar intervertebral disc herniation, serious complications have been reported. Accordingly, alternative substances for chemonucleolysis have been sought. The main beneficial effect of chemonucleolysis derives from the decrease in intradiscal pressure. Several previous studies have investigated the relationship between physiological saline injection and disc mechanics in cadaveric specimens [2, 5, 16]. However, no previous study has assessed the intradiscal pressure after intradiscal injection of “hypertonic saline” in living animals. The present study compared the changes in intradiscal pressure after intradiscal injection of hypertonic saline with those after chymopapain injection. The lumbar intervertebral discs of 26 living rabbits were examined: 10% hypertonic saline was injected

in ten rabbits, and chymopapain (10 pikokatal units) was injected intradiscally in another ten, with the remaining six being used as controls. The intradiscal pressure was measured at 1, 4, and 12 weeks after injection. The intradiscal pressure of the hypertonic saline-injected group at 4 weeks was significantly lower than that of the control group, but by 12 weeks it had recovered. On the other hand, that of the chymopapain-injected group remained significantly lower than that of the control group at 12 weeks. The results of this study found that hypertonic saline injected into the intervertebral discs temporarily decreased the intradiscal pressure.

Key words Chemonucleolysis · Chymopapain · Hypertonic saline · Intradiscal injection · Intradiscal pressure

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Introduction

Chemonucleolysis was first suggested as a possible treatment for low-back pain and sciatica by Hirsch [7]. Since the first clinical use of chymopapain by Smith [19], this technique has been widely employed in Canada and Europe. In 1982, its approval by the Food and Drug Administration made it available as an alternative treatment for intervertebral disc herniation in the United States. Its mechanism has been considered to be through decreasing intradiscal pressure by rapid hydrolysis of the proteogly-

can of the nucleus pulposus [9, 10, 21]. Its success rate has been reported as 60–90% [11, 13, 14, 20]. Complications such as anaphylactic reaction, paralysis, and long-term back pain or spasm have been observed in some cases. One frequently encountered problematic complication has been the anaphylactic reaction, in which various symptoms such as erythematous rash, urticaria, pruritus and shock are manifested. This reaction occurs in 0.35–0.9% of patients [6, 11, 14]. To prevent anaphylactic reaction, skin testing of chymopapain before injection has been recommended, but the usefulness of skin testing in reducing the risk to anaphylaxis has remained controver-

sial. Accordingly, an alternative substance to of chemonucleolysis has been sought.

Proteoglycans are major structural components of the intervertebral disc, and their concentration and organization in the extracellular matrix have a considerable influence on its mechanical properties. Bayliss et al. [3] have described a reduction in proteoglycan synthesis rates when the disc hydration was decreased. Shioda [18] suggested a mechanism for a decrease in the intradiscal pressure after hypertonic saline injection, by which partial cell necrosis leads to a decrease in matrix production. Released lysosomal enzymes from the cells could then cause partial proteolysis. As a result, the amount of proteoglycan decreases, namely, the water-binding capacity decreases. He suggested that the direct effect of hypertonic saline on the matrix was minimal. However, there has not yet been any quantitative pressure measurements with various dosages of saline, and no studies over time.

The present study examined the effect of intradiscal injection of 10% hypertonic saline into intervertebral discs of living rabbits.

Materials and methods

Twenty-six male Japanese white rabbits (body weight 2.5–3.0 kg, age 4–6 months) were examined in this study. Thirty lumbar intervertebral discs in six rabbits were used as controls without injection. Five lumbar discs from L1/2 to L5/6 of ten rabbits were injected with 500 pikokatal units (pku)/ml of chymopapain, and those of the other ten were injected with 10% hypertonic saline. A dosage of 0.02 ml of each substance was injected under open surgery through the lateral approach using a 23-G needle and a 1-ml syringe for each disc, under general anesthesia by intravenous injection of sodium pentobarbital (10–15 mg/kg body weight) and ketamine hydrochloride (30–50 mg/kg body weight). Chymopapain and/or hypertonic saline injection did not lead to persistent pain or to increased muscle activity in animals. Three rabbits in each injected group were euthanized by intravenous injection of an overdose of pentobarbital at 1 week, 4 weeks, and 12 weeks after the intradiscal injection, to measure the intradiscal pressure. The six rabbits in the control group were all euthanized at 1 week. The spines were then immediately removed from the bodies and the intradiscal pressure in the prone position was measured within 1 min. The control and injected discs were not obtained from the same animals, because we had to exclude the influence of disc degeneration following needle puncture. One rabbit in each group at 24 weeks was euthanized for histological investigation. Intradiscal pressure was recorded using an amplifier (Model 8805 C Pressure Amplifier, Hewlett Packard, Andover, Mass., USA) and recorder (Thermal Array Recorder RTA1300 M, Nihon Koden, Tokyo, Japan). A 23-G needle (0.65 mm diameter) was used for the pressure measurement. One end of the tube (Arterial pressure tubing-SL, Abbott Ireland, Sligo, Ireland) filled with normal saline was connected to a 23-G needle, and a pressure transducer (Model 1290 A, Hewlett Packard) was connected to this tube at the other side. The tube was flushed with 1 ml normal saline, and a zero balance was adjusted before pressure measuring.

The extracted spines were then fixed in 20% formalin for 1 week, and washed in running water for 12 h, after which they were decalcified in Plank & Rychlo solution over 2 weeks. Each decalcified specimen was opened by a midline incision (sagittal section) to observe changes in the nucleus pulposus, in the annulus

Fig. 1 Intradiscal pressure in the control group, and at different times after intradiscal injection of 10% hypertonic saline or 10 pikokatal units (pku) chymopapain

fibrosus and in the disc height; this was followed by dehydration in alcohol, clearance in chloroform, and then embedding in paraffin. The specimens were then thin-sectioned at 5 μ m and subjected to hematoxylin and eosin staining for histological investigation of the discs.

Results

Intradiscal pressure

All data were presented as mean($\pm$ SD). $P < 0.05$ was used as the level of statistical significance. The period of this experiment, 12 weeks, was comparatively short in terms of the life-span of these animals. We therefore believed that the intradiscal pressure of the non-injected discs (controls) did not change over the period of this experiment.

Statistical comparison between groups was performed by three-way ANOVA (analysis of variance) followed by the GLM (general linear models) procedure [17]. There

Fig. 2A,B Longitudinal section of the decalcified specimen of hypertonic saline-injected disc. The nucleus pulposus had a normal viscous appearance, and the disc height was maintained until 24 weeks. **A** At 4 weeks after injection, and **B** at 24 weeks after injection

Fig. 3A,B Longitudinal section of the decalcified specimen of chymopapain-injected disc. The nuclear space was occupied by a creamy substance, and the disc height continued reducing until 24 weeks. **A** At 4 weeks after injection, and **B** at 24 weeks after injection

Fig. 4A,B Histological changes in the specimen at 24 weeks following injection of hypertonic saline. **A** Necrotic cells were observed in the central portion of the nucleus pulposus (hematoxylin and eosin stain, original magnification $\times 5$). **B** Halos were observed surrounding nucleus pulposus cells, with vacuoles (hematoxylin and eosin stain, original magnification $\times 25$)

Fig. 5A, B Histological changes in the specimen at 24 weeks following injection of chymopapain. **A** The posterior inner part of the annulus fibrosus proliferated toward the central portion of the disc (hematoxylin and eosin stain, original magnification $\times 5$). **B** The nucleus pulposus was partially replaced by regenerated fibrocartilaginous tissue (hematoxylin and eosin stain, original magnification $\times 25$)

was a significant difference between the three groups (control, 10% hypertonic saline and chymopapain). However, there was no significant difference according to the passage of time (1 week, 4 weeks and 12 weeks) or between the sites (each disc from L1/2 to L5/6).

A *t*-test (Fisher's least significant difference) was used for comparison of the pressure within each injected group with that of the control group [17]. The mean value of the intradiscal pressure measured in the control group was 18.2 ± 7.3 mmHg ($n = 6$, 30 discs). There was no significant difference between the intradiscal pressure of the hypertonic saline-injected discs measured at 1 week (15.4 ± 10.4 mmHg, $n = 3$, 15 discs) and the control discs. However, the pressure of the hypertonic saline-injected discs was significantly lower at 4 weeks (11.7 ± 4.8 mmHg, $n = 3$, 15 discs). By 12 weeks, the mean value of the pressure was at the same level as that of the control group (18.0 ± 11.5 mmHg at 12 weeks, $n = 3$, 15 discs).

The mean value of the intradiscal pressure of the chymopapain-injected discs was significantly lower than that of the control group at 1 week (3.4 ± 3.1 mmHg, $n = 3$, 15 discs), and was lowest at 4 weeks (1.5 ± 1.8 mmHg, $n = 3$, 12 discs). However, at 12 weeks, it was slightly higher than at 4 weeks, but still significantly lower than that of the control group (4.3 ± 1.4 mmHg, $n = 3$, 15 discs) (Fig. 1).

Macroscopic examination

The gross appearance of a specimen hypertonic saline-injected disc is shown in Fig. 2. The border between the nucleus pulposus and the internal annulus fibrosus was clearly defined at 4 weeks, and at 24 weeks. The fibers of the internal annulus fibrosus were arranged in an orderly fashion. The nucleus pulposus had a predominantly normal viscous appearance, and the disc height was maintained until 24 weeks, and was partially occupied by a creamy substance.

In the chymopapain-injected disc, in contrast, at 4 weeks after injection, the nuclear space was fully occupied by the creamy substance. There was disturbance in the fiber array of the internal annulus fibrosus. The border between the nucleus pulposus and the internal annulus fibrosus was unclear, and the disc height was decreased in the specimen at 24 weeks compared to the hypertonic saline-injected discs (Fig. 3).

Microscopic examination

With low-magnification light microscopy, necrotic cells were observed in the central portion of the nucleus pulposus at 4 weeks following the hypertonic saline injection. Under high magnification, halos surrounding nucleus pulposus cells were partially observed at 4 weeks, but nucleus pulposus cells remained until 24 weeks (Fig. 4).

In the chymopapain-injected discs under low magnification, the disc height was markedly decreased and the nucleus pulposus had disappeared at 1 week. The posterior inner part of the annulus fibrosus had proliferated toward the central portion of the disc (inward bulging), and filled the space from which the nucleus pulposus had disappeared. At 24 weeks, the space was fully occupied by regenerated fibrocartilaginous tissue, and disc space narrowing was maintained (Fig. 5).

The effect of hypertonic saline was partial necrosis of the nucleus pulposus. In contrast, chymopapain disrupted not only the nucleus pulposus, but also the annulus fibrosus.

Discussion

There have been several reports concerning intradiscal pressure involving chymopapain-injected disc [4, 12, 15, 22] and physiological saline-injected disc [2, 5, 16]. However, there has been no report on the intradiscal pressure after injection of hypertonic saline in living animals.

Several recent papers describing intradiscal pressure measurements reported that good results were obtained using strain-gauge technology [1, 23]. The diameter of the needles in the reported studies was 1.3–1.5 mm – too large for using in our study, because the disc height of the rabbits injected with chymopapain was smaller than that. We therefore used a very small-diameter needle (0.65 mm), which required us to use a fluid-coupled device. We employed a traditional simple liquid-coupled system [15], which has been clearly found to demonstrate the hydrostatic features of the central region of the disc without the necessity for a special needle.

Studies aimed at investigating whether near-maximal degradation in the human intervertebral disc could be achieved with a dosage lower than that currently recommended for chemonucleolysis seem to be warranted. Kiester et al. [8] reported the dosage-related effect of intradiscal chymopapain on rabbit intervertebral discs. We injected only 10 pku of chymopapain, less than was used in their study (20–4000 pku). In the present study, the intradiscal pressure was significantly decreased and the disc height was reduced. Moreover, if an excellent response can be obtained with a lower dosage than usually recommended, there is the potential for effective chemonucleolysis with fewer side-effects. Side-effects after injection of chymopapain occur because chymopapain is a foreign protein.

Clinically, it is important to find safer and more reliable agents that can decrease intradiscal pressure without causing significant damage to the structure of the discs, because the purpose of intradiscal injection is not to destroy the discs. Moreover, keeping disc destruction to a minimum may promote repair of the disc tissue.

Since hypertonic saline is not a foreign protein, it is physiologically and clinically safer than chymopapain or

any other foreign protein. We showed that injecting hypertonic saline into the lumbar discs of living rabbits reduced the intradiscal pressure after 4 weeks, but not after 12 weeks. In contrast, the injection of chymopapain caused a larger and more persistent reduction in pressure, and also disrupted the annulus fibrosus. These findings suggested that hypertonic saline injections may be clinically useful, because they can disrupt chondrocyte metabolism in the nucleus by a sufficient amount to cause tem-

porary decompression, without causing the severe disruption associated with chymopapain. We expect that chemonucleolysis using alternative substances may become an effective, minimally invasive treatment, in place of open surgery, percutaneous nucleotomy or chemonucleolysis using chymopapain.

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