Kimiaki Sato
Kensei Nagata
Teruyuki Hirohashi

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

Received: 28 April 2000
Revised: 23 December 2000
Accepted: 12 January 2001
Published online: 7 March 2001
© Springer-Verlag 2001

K. Sato (✉) · K. Nagata · T. Hirohashi
Department of Orthopaedic Surgery,
Kurume University School of Medicine,
67 Asahi-machi, Kurume 830-0011, Japan
e-mail: spine@med.kurume-u.ac.jp,
Tel.: +81-942-317568,
Fax: +81-942-350709

Abstract Chemonucleolysis with chymopapain is an effective alternative to an operation for the treatment of some patients who have a lumbar intervertebral disc herniation. However, chymopapain is associated with rare but serious complications. Accordingly, alternative substances for chemonucleolysis have been sought. The main beneficial effect of chemonucleolysis derives from the decrease in the intradiscal pressure. We have previously reported that hypertonic saline injected into the intervertebral discs decreased the intradiscal pressure, but only temporarily. The present experimental study investigated changes in the intradiscal pressure after a repeat intradiscal injection of hypertonic saline. The lumbar intervertebral discs of 18 living rabbits were examined: 10% hypertonic saline was injected intradiscally just

once in 12 rabbits, and the same dosage was injected again, 4 weeks later, in the same animals. The intradiscal pressure was measured at 1, 4, 8, and 12 weeks after the second injection. The remaining six rabbits were used as controls, without puncture and without injection. The intradiscal pressure of the group with repeat hypertonic saline injection at 4 weeks was significantly lower than that of the control group. The decreased pressure showed a tendency to increase at 8 weeks, and it had recovered at 12 weeks. The results of this study suggest that repeat hypertonic saline injections may be clinically useful.

Keywords Chemonucleolysis · Hypertonic saline · Intradiscal pressure · Repeat intradiscal injection

Introduction

Chemonucleolysis with chymopapain, which was first clinically used by Smith [12], is an effective treatment for intervertebral disc herniation. However, anaphylactic reactions, in particular, have limited the use of this treatment. To prevent anaphylactic reactions, skin testing and the use of H1 and H2 blocking agents have been recommended. Anaphylactic reactions to chymopapain have decreased in frequency over the years [5]. However, the risk of anaphylaxis still remains. Accordingly, an alternative substance for chemonucleolysis has been sought.

Proteoglycans are major structural components of the intervertebral disc, and their concentration and organiza-

tion in the extracellular matrix have a considerable influence on its mechanical properties. Bayliss et al. [2] described a reduction in proteoglycan synthesis rates when disc hydration was decreased.

Shioda [11] 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, which means the water-binding capacity decreases. He suggested that the direct effect of hypertonic saline on the matrix was minimal.

We have previously reported intradiscal pressure after intradiscal injection of hypertonic saline in rabbits, but the results of the study found that hypertonic saline injected

into the intervertebral discs only temporarily decreased the intradiscal pressure [10]. The present study investigated the effect of a repeat intradiscal injection of 10% hypertonic saline into the intervertebral discs of rabbits.

Materials and methods

Eighteen 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 puncture and without injection. Five lumbar discs from L1/2 to L5/6 of 12 rabbits were injected with 10% hypertonic saline just once, and the same animals were injected with hypertonic saline again at 4 weeks after the first injection. A dosage of 0.02 ml hypertonic saline 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). Three rabbits were sacrificed by an intravenous injection of an overdose of pentobarbital at 1, 4, 8, and at 12 weeks after the second intradiscal injection, to measure the intradiscal pressure. The discs were not obtained from just a single animal, because we had to exclude the influence of disc degeneration following needle puncture. Six rabbits in the control group were all sacrificed at 1 week. The spine was immediately removed and the intradiscal pressure in the prone position was measured within 1 min.

Intradiscal pressure was recorded using an amplifier (Model 8805C Pressure Amplifier, Hewlett Packard, Andover, Mass., USA) and recorder (Thermal Array Recorder RTA1300 M, Nihon Kodens, 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 1290A, Hewlett Packard) was connected to this tube at the other end. The tube was flushed with 1 ml normal saline, and a zero balance was adjusted before pressure measuring. Multiple measurements were recorded for each disc.

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 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. This study was approved by the Committee of Animal Experimentation, Kurume University School of Medicine.

Statistical analysis of results

Mean values ($\pm$ SD) are presented for all results. For each parameter, comparisons between groups of data were made using a two-way analysis of variance followed by the general linear models (GLM) procedure [8], assuming a null hypothesis. If differences between groups were apparent, the raw data were examined using a *t*-test (Fisher's Least Significant Difference) between the pressure in the injected group and that of the control group [8]. Statistical significance was considered to occur at $P < 0.05$.

Fig. 1 Intradiscal pressure in the control group, and at different times after repeat intradiscal injection of 10% hypertonic saline

Fig. 2A,B Longitudinal section of the decalcified specimens. **A** A control disc. Nucleus pulposus showed a viscous appearance, and fibers were arranged in an orderly fashion in the internal annulus fibrosus (bar=5 mm). **B** At 12 weeks after repeat hypertonic saline injection, the border between the nucleus pulposus and the internal annulus fibrosus was clearly defined. The nucleus pulposus had a predominantly normal viscous appearance, and was partially occupied by a creamy substance. The disc height was maintained at 12 weeks (bar=5 mm)

Fig. 3A,B A control disc. **A** The nucleus pulposus was surrounded by the annulus fibrosus, which was composed of lamellar fibrous tissue (hematoxylin and eosin stain, original magnification $\times 5$). **B** The same specimen under high magnification of the nucleus pulposus. There were many notochordal cells in the nucleus pulposus. These were identified as ring-like aggregates of vacuolated cells among the ground substance (hematoxylin and eosin stain, original magnification $\times 20$)

Results

Intradiscal pressure

The mean value of the intradiscal pressure measured in the control group was 18.7 ± 8.3 mmHg ($n=6$, 30 discs). There was no significant difference between the intradiscal pressure of the hypertonic saline-injected discs measured at 1 week (18.6 ± 8.6 mmHg, $n=3$, 10 discs) and that of the control discs. However, the pressure of the hypertonic saline-injected discs was significantly lower at 4 weeks (9.7 ± 3.8 mmHg, $n=3$, 15 discs), and showed a tendency to recovery at 8 weeks (14.7 ± 5.0 mmHg, $n=3$, 13 discs). By 12 weeks, the mean value of the pressure was at the same level as that of the control group (22.5 ± 6.9 mmHg, $n=3$, 15 discs) (Fig. 1).

Fig. 4A,B A repeat hypertonic saline-injected disc. **A** No degenerative changes were shown in the internal annulus fibrosus and cartilaginous end plate at 12 weeks (hematoxylin and eosin stain, original magnification $\times 5$). **B** The same specimen under high magnification of the nucleus pulposus. Nucleus pulposus cells remained, but the ring-formed aggregations of these cells were collapsed, and halos surrounding nucleus pulposus cells with vacuole were observed (hematoxylin and eosin stain, original magnification $\times 20$)

Macroscopic examination

The gross appearance of a control disc is shown in Fig. 2A. The nucleus pulposus shows a viscous appearance, and fibers are arranged in an orderly fashion in the internal annulus fibrosus. This is in contrast to the gross appearance of a disc 12 weeks following a second hypertonic saline injection (Fig. 2B), where the border between the nucleus pulposus and the internal annulus fibrosus is clearly defined. The fibers of the internal annulus fibrosus are arranged in an orderly fashion, while the nucleus pulposus has a predominantly normal viscous appearance, and is partially occupied by a creamy substance. The disc height was maintained at 12 weeks.

Microscopic examination

In a control disc, the nucleus pulposus was surrounded by the annulus fibrosus, which was composed of lamellar fibrous tissue. There were many notochordal cells in the nucleus pulposus. These were identified as ring-like aggregates of vacuolated cells among the ground substance (Fig. 3).

In a second hypertonic saline-injected disc, nucleus pulposus cells remained at 12 weeks, but the ring-formed aggregations of these cells were collapsed, and halos surrounding nucleus pulposus cells with vacuole were observed. These findings suggested degeneration of notochordal cells. No degenerative histological change was seen in the internal annulus fibrosus or in the cartilaginous end plate (Fig. 4).

Discussion

There have been several reports concerning intradiscal pressure after physiological saline injection [1, 3, 7]. However, there has been no report on intradiscal pressure after repeat injection of hypertonic saline.

Several recent papers describing intradiscal pressure measurements reported that good results were obtained using strain-gauge technology [9, 13]. The diameter of the needles in the reported studies was 1.2–1.5 mm – too large for using in our study. We therefore used a very small diameter needle (0.65 mm), which required us to use a simple liquid-coupled system.

A nuclear material does not always exhibit hydrostatic behavior [4]. The pressure in a severely degenerated disc cannot, therefore, be measured exactly using a liquid-coupled system, but the system can show whether the pressure decreases or not. It is a well-known fact that the pressure in water is transmitted evenly in every direction according to Pascal's principle. As the hypertonic saline injected discs are mildly degenerate histologically, the discs still include a large quantity of free water. We therefore employed a traditional simple liquid-coupled system [6], which has been clearly found to demonstrate the hydrostatic features of the central region of the disc without the need for a special needle.

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 to preserve the discs. Moreover, keeping disc destruction to a minimum may promote repair of the disc tissue.

We have previously reported that hypertonic saline injected just once into the intervertebral disc temporarily decreased the intradiscal pressure for 4 weeks, while the injection of chymopapain caused a relatively larger and more persistent reduction in pressure [10]. Therefore, we injected hypertonic saline once again at 4 weeks after the first injection. The pressure had recovered at 1 week after the repeat injection. This implied that the decreasing pressure at 4 weeks after the first injection may have recovered within 1 week. This is probably because the active period of hypertonic saline is short. But if repeat hypertonic saline injection is done before recovery in pressure, then a decrease in intradiscal pressure could be sustained. Since hypertonic saline is not a foreign protein, it is physiologically and clinically safer than chymopapain or any other foreign protein.

In the group with repeat hypertonic saline injection, the intradiscal pressure was significantly lower than that in the control group, and repeat hypertonic saline injections did not cause severe disruption compared with chymopapain, histologically. These findings suggest hypertonic saline may be clinically and functionally useful. Though the present study showed that 0.02 ml 10% hypertonic saline certainly has the potential for reducing intradiscal pressure, an injection of more than twice that amount and/or in higher concentration may be more effective clinically. We expect that chemonucleolysis using hypertonic saline may become an effective minimally invasive treatment, in place of open surgery, percutaneous nucleotomy or chemonucleolysis using chymopapain.

Acknowledgements The authors thank Associate Professor Hiroki Inutsuka, Computer Center for Medical Research, Computer Education Center, Kurume University, for the helpful advice provided during statistical analysis of the data. We also thank Mr. Kazuhiro Yoshida for his technical assistance.

References

- Andersson GBJ, Schultz AB (1979) Effects of fluid injection on mechanical properties of intervertebral discs. *J Biomech* 12:453–458
- Bayliss MT, Johnstone B, O'Brien JP (1988) Proteoglycan synthesis in the human intervertebral disc. Variation with age, region and pathology. *Spine* 13:972–981
- Dolan P, Adams MA, Hutton WC (1987) The short-term effects of chymopapain on intervertebral discs. *J Bone Joint Surg Br* 69:422–428
- Nachemson A (1960) Lumbar intradiscal pressure. *Acta Orthop Scand* 43 [Suppl]:1–104
- Nordby EJ, Wright PH, Schofield SR (1993) Safety of chemonucleolysis. Adverse effects reported in the United States, 1982–1991. *Clin Orthop* 293: 122–134
- Quinnell RC, Stockdale HR, Willis DS (1983) Observations of pressure within normal discs in the lumbar spine. *Spine* 8:166–169

-
7. Ranu HS (1993) Multipoint determination of pressure-volume curves in human intervertebral discs. *Ann Rheum Dis* 52:142–146
 8. SAS Institute (1990) SAS/STAT user's guide, version 6, vol 2, 4th edn. SAS Institute, Cary, pp 891–996
 9. Sato K, Kikuchi S, Yonezawa T (1999) In vivo intradiscal pressure measurement in healthy individuals and in patients with ongoing back problems. *Spine* 24:2468–2474
 10. Sato K, Nagata K, Ariyoshi M, Hirohashi T, Inoue A (2000) Intradiscal pressure after intradiscal injection of hypertonic saline: an experimental study. *Eur Spine J* 9:213–217
 11. Shioda M (1995) Intradiscal injection of hypertonic saline, phenol-glycerin and osmic acid for the treatment of lumbar disc herniation: an experimental study [English abstract]. *J Jpn Orthop Assoc* 69:964–976
 12. Smith L (1964) Enzyme dissolution of the nucleus pulposus in humans. *JAMA* 187:137–140
 13. Wilke H-J, Neef P, Caimi M, Hoogland T, Claes LE (1999) New in-vivo measurements of pressures in the intervertebral disc in daily life. *Spine* 24:755–762