Received: 10 March 2022 | Revised: 22 June 2022 | Accepted: 24 July 2022

DOI: 10.1002/jsp2.1221

RESEARCH ARTICLE

JOR Spine OPEN ACCESS

Effect of contrast media on the enzyme activity of condoliase: In vitro assessment

Ippei Watanabe[1] | Taiichi Shirogane[1] | Yukihiro Matsuyama[2] | Kazuhiro Chiba[3]

[1]Medical Affairs, Seikagaku Corporation, Tokyo, Japan

[2]Department of Orthopaedic Surgery, Hamamatsu University School of Medicine, Shizuoka, Japan

[3]Department of Orthopaedic Surgery, National Defense Medical College, Saitama, Japan

Correspondence
Ippei Watanabe, Medical Affairs, Seikagaku Corporation, 1-6-1 Marunouchi, Chiyoda-ku, Tokyo 100-0005, Japan.
Email: ippei.watanabe@seikagaku.co.jp

Abstract

Background: Condoliase has been used in Japan to treat patients with lumbar disc herniation by its injection into the nucleus pulposus. The injection of condoliase together with contrast media is prohibited; because there are no data whether contrast media have any effect on condoliase activity. This study aimed to elucidate the effects of contrast media on condoliase activity.

Methods: Condoliase with chondroitin sulfate (CS) and without CS were mixed with various contrast media (nonionic [iohexol or iotrolan]; ionic [amidotrizoic acid]). (i) The mixtures with CS were incubated at 37°C; (ii) the mixtures without CS were stored at 24°C for 60 min, followed by addition of CS to assess condoliase activity by measuring the amount of *N*-acetylhexosamines enzymatically cleaved from CS using Morgan–Elson method.

Results: (i) In the presence of CS, the ionic contrast media reduced condoliase activity within 10 min in a dose-dependent manner, and the nonionic contrast media had no effect on condoliase activity for at least 120 min. (ii) In the absence of CS, the ionic contrast media almost completely inactivated condoliase within 15 min, and the nonionic contrast media also reduced condoliase activity; the residual activity was 65% with iotrolan and 35% with iohexol at 60 min.

Conclusions: The ionic contrast media significantly reduced condoliase activity regardless of presence or absence of CS. Although the nonionic contrast media did not affect condoliase activity in the presence of CS, it reduced activity in the absence of CS. Mixing condoliase with contrast media, especially ionic type contrast media, should be avoided.

KEYWORDS

chondroitin sulfate, condoliase, contrast media, lumbar disc herniation, Morgan–Elson method

1 | INTRODUCTION

Lumbar disc herniation (LDH) is caused by extrusion of the nucleus pulposus through the posterior annulus fibrosus into the spinal canal leading to compression of the spinal nerve roots, causing back and leg pain.[1] Primary treatment option for LDH is conservative, and when conservative managements fail, surgery is the choice, which, however, is often associated with risks of complications and reoperation.[2]

In the late 1960s to 1970s, an enzyme-based treatment for LDH, commonly known as chemonucleolysis, prevailed mainly in the

United States and Europe as an intermediate option lying between conservative and surgical treatment. The procedure involved direct injection of a proteolytic enzyme chymopapain into the intervertebral disc by a single needle puncture under local anesthesia, leading to improvement of symptoms by dissolution of the nucleus pulposus.[3,4] Chemonucleolysis with chymopapain is less invasive and provided comparable outcomes with that of surgery if patients were chosen properly.[1,5–7] Unfortunately, chymopapain caused various adverse events such as anaphylaxis, paraplegia, and back spasm mostly due to its proteolytic activity, affecting tissues surrounding the intervertebral disc, and was gradually withdrawn from the market.[8,9]

Condoliase derived from a gram-negative rod, *Proteus vulgaris*, was initially used for a pure chemical purpose as a glycosaminoglycan-degrading enzyme.[10,11] Unlike chymopapain, condoliase lacks proteolytic activity and specifically degrades chondroitin sulfate (CS), which is abundant in the nucleus pulposus of the intervertebral disc.[11–13] Iwata, who was then engaged in basic analytical researches on articular cartilage using condoliase, noticed that condoliase could overcome the drawbacks of chymopapain and be an effective alternative treatment for LDH by reducing intradiscal pressure and the volume of herniation by degrading CS without affecting peridiscal tissues including nerves and vessels.[14–16] He started a project aiming clinical application of condoliase and a series of basic research studies and clinical trials, condoliase was approved by the Ministry of Health, Welfare and Labor in 2018 in Japan, 50 years after its initial conception by Iwata. Its best indication is for a patient with subligamentous extrusion type of LDH, whose symptoms are refractory to conservative treatments, and favorable clinical results with approximately 80% success have been reported.[15–17] As of December 2021, more than 4300 patients had been enrolled in the postmarketing safety surveillance of condoliase. Most frequent adverse events were skin rash and urticaria, transient back and leg pain that were observed in approximately 10% of the patients. There were no deaths or occurrences of paraplegia and severe back spasm as reported with chymopapain. Currently, a phase III trial is ongoing in the United States for FDA approval.[18]

Magnetic resonance imaging (MRI) is a standard noninvasive imaging modality for assessment of LDH. In some cases, however, lumbar discography is more effective than MRI for the diagnosis of LDH.[19–21] Lumbar discography is especially useful in discriminating whether LDH is subligamentous or transligamentous by depicting the consistency of the posterior longitudinal ligament. Iodinated contrast media are commonly used for discography, which are classified into two types, ionic and nonionic, based on their water solubility.[19,20] All of the iodinated contrast media have a tri-iodinated benzene ring as a basic structure. Compounds of the ionic type, containing the benzene ring substituted with a carboxylate (—COOH), dissociate in solution and therefore have a high osmolality. For example, ionic contrast media containing amidotrizoic acid have osmolality about six times that of the blood. On the other hand, nonionic contrast media contain benzene rings without a carboxylate functional group that are more hydrophilic than ionic compounds, resulting in much lower osmolality.[6,22]

The use of condoliase together with contrast media into the lumbar disc is prohibited, because (i) there are no data on the stability, efficacy, and safety of condoliase when mixed with other agents; (ii) it is not clear if its concomitant use with contrast media induces serious neurological complications, for example, transverse myelitis, paraplegia, cerebral hemorrhage, and so on.[9] If these two can be used combined, the surgeon can inject contrast medium first to check if the position of the needle tip is correctly placed inside the NP and confirm the diagnosis of subligamentous extrusion, and then inject condoliase with confidence.

In the current study, we have investigated if contrast media have any effect on the activity of condoliase when these two are mixed, because there is no data on this respect. The results of the present in vitro study may serve as basis to further investigate the compatibility of the two regarding safety, efficacy, and pharmacokinetics for future clinical application.

2 | MATERIALS AND METHODS

2.1 | Materials

Condoliase and chondroitin sulfate (CS) extracted from shark cartilage were obtained from Seikagaku Corp. (Tokyo, Japan). The contrast media used in this investigation are listed in Table 1. Omnipaque® 180, 240, and 300 were purchased from Daiichi Sankyo Company Ltd. (Tokyo, Japan), hereafter, referred to as iohexol-180, -240, and -300, respectively. Isovist® 240 and Urografin® 60% were purchased from Bayer Yakuhin, Ltd. (Osaka, Japan) hereafter, referred to as iotrolan-240 and amidotrizoic acid-292, respectively.

2.2 | Optimization of enzyme concentration and reaction time in this study by Morgan–Elson method

To investigate the optimal concentration of condoliase for this study, dilutions of condoliase (0.01–5.0 U/ml) were prepared in 50 mM Tris–HCl buffer (pH 8.0). Each condoliase solution (30 μl) was mixed with an equal volume of 0.9% NaCl. Subsequently, CS solution was added to the mixture to make the final concentration of CS to 0.075%. Condoliase activity was measured by the Morgan–Elson method, as follows: (i) the mixture was incubated at 37°C for 10 min; (ii) the mixture was heated to 100°C for 1 min to stop the enzymatic reaction; (iii) to generate chromogen, 100 μl of 0.5% $K_2B_4O_7$ (pH 9.0) was added, and the mixture was heated to 100°C for 7 min; (iv) color development was initiated by adding 1 ml of acetic acid and 0.4 ml of Ehrlich's reagent (16 w%/v% *p*-dimethylaminobenzaldehyde in a mixture solution with 95 v%/v% acetic acid and 5 v%/v% hydrochloric acid), and then incubating for 20 min at 37°C; Ehrlich's reagent reacted with a chromogen formed

TABLE 1 Contrast media used in this study

Classification	Brand name	Active ingredient (mg/ml)	Iodine content (mg/ml)	Physical property [a]	
				Viscosity (mPa s)	Osmotic pressure
Ionic type	Urografin® 60%	Amidotrizoic acid (471.8 mg/ml)	292	3.83–4.17	6
Nonionic type	Omnipaque® 180	Iohexol (388.2 mg/ml)	180	2.0	1
	Omnipaque® 240	Iohexol (517.7 mg/ml)	240	3.3	2
	Omnipaque® 300	Iohexol (647.1 mg/ml)	300	6.1	2
	Isovist® 240	Iotrolan (512.6 mg/ml)	240	3.9	1

[a]Information in the package insert of each product as of March 2022.

from *N*-acetylhexosamines enzymatically cleaved from CS to give a reddish-purple colored product; and (v) the absorbance of the reaction solution was measured at 585 nm using a spectrophotometer (UV-1900i, Shimadzu, Kyoto, Japan).[10,23]

2.3 | Measurement of condoliase activity in a mixture with contrast media and CS

30 μl of 0.3 U/ml condoliase solution was mixed with different amounts of contrast media as follows: an equal, double, and three-times the volume of the condoliase solution. CS was then added to the mixture of condoliase and contrast media to reach a final concentration of 0.075%. The volumes and concentrations of CS solutions added to various samples in individual runs are shown in Table S1. To assess the effect of osmotic pressure, 6% NaCl solution was also mixed with condoliase. The residual activity (%) of condoliase mixed with contrast media or NaCl was measured by Morgan–Elson method as described above, and expressed as the absorbance of the sample without contrast media set as 100%.

In addition, to evaluate the effect of the exposure time of contrast media on condoliase activity, 30 μl of 0.03 U/ml condoliase solution was mixed with an equal volume of nonionic contrast media in the presence of CS, and the mixture was incubated at 37°C in the dark for 30, 60, and 120 min. After incubation, condoliase activity was measured by Morgan–Elson method. The experimental conditions are summarized in Table S2.

2.4 | Measurement of condoliase activity in a mixture with contrast media but without CS

30 μl of 0.3 U/ml condoliase solution was mixed with an equal volume of nonionic contrast media or saline without adding CS. Each mixture was stored at 0, 15, 30, and 60 min, in the dark at room temperature (24°C). After storage, CS solution was added to the mixture to make a final CS concentration of 0.075%. The condoliase activity in each sample was measured using Morgan–Elson method. The residual condoliase activity (%) at each time point was calculated according to the following formula: (absorbance at each time point/ absorbance at time 0) × 100. The experimental conditions are summarized in Table S2. Moreover, to evaluate the influence of temperature on condoliase activity, condoliase (0.3 U/ml) in saline without CS was stored for 60 min in the dark at 37°C.

2.5 | Statistical analysis

In the measurements of the condoliase activity, the Satterthwaite *t*-test assuming unequal variances was used to compare different mixtures (1:1 and 1:2 or 1:3) of condoliase and contrast media (Section 2.3, Table S1). Residual activity of condoliase mixed with contrast medium was compared with that of condoliase alone at each time point using Dunnett's test for multiple comparison (Sections 2.3 and 2.4). *p* values of less than 0.05 were considered significant.

3 | RESULTS

3.1 | Characteristics of contrast media and determination of a suitable method to measure activity

All iodinated contrast media (iohexol, iotrolan, and amidotrizoic acid) have absorption peaks in the spectrum range of 200–300 nm because of the benzene ring of the active ingredient (Figures S1 and S2). Condoliase cleaves the glycosidic bond at the nonreducing end of CS, creating a C4—C5 double bond in uronic acid that absorbs ultraviolet light at 232 nm. Therefore, absorbance at 232 nm of the cleaved CS has routinely been used to measure condoliase activity.[24] The results of Figures S1 and S2 suggested that this conventional method could not be used when condoliase was mixed with contrast media. The Morgan–Elson method using absorbance at the different wavelength of 585 nm was therefore employed to quantify the *N*-acetylhexosamines enzymatically cleaved from CS.

3.2 | Optimization of enzyme concentration and reaction time

As shown in Figure 1A,B, condoliase with a concentration of 0.01–0.6 U/ml was positively and linearly correlated with the

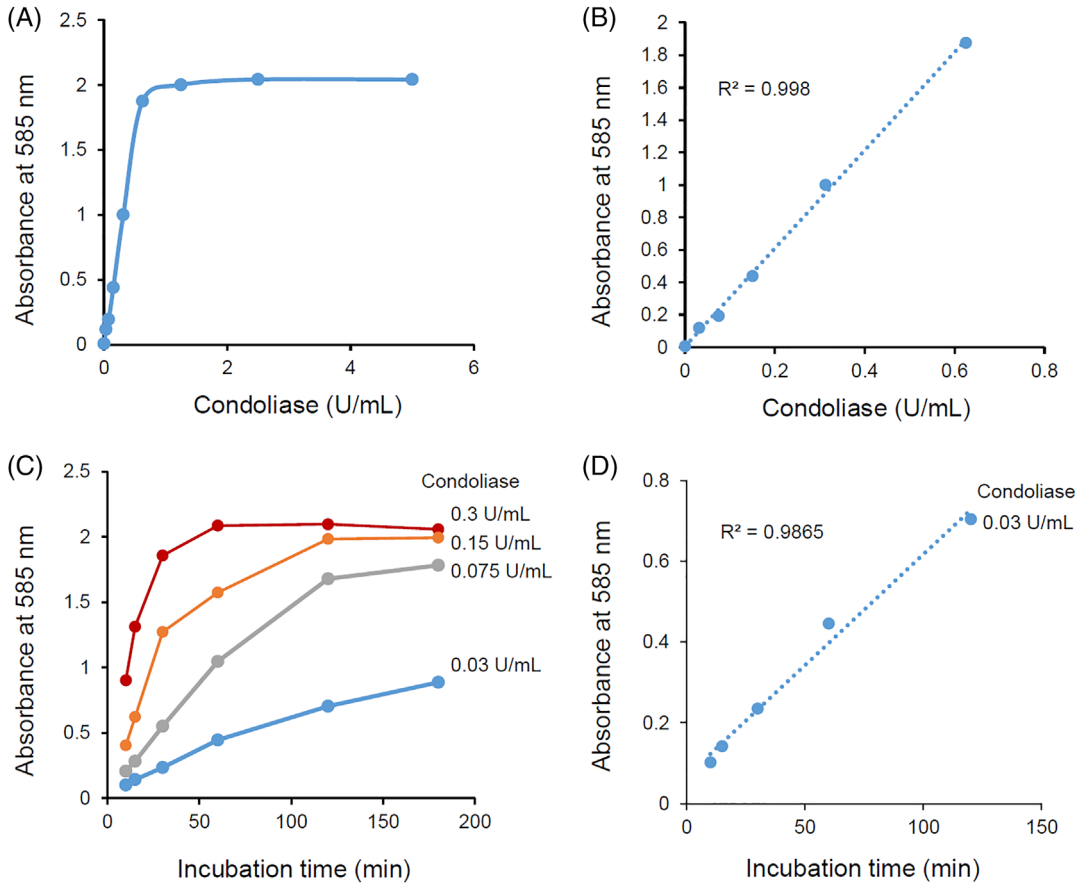

FIGURE 1 Optimization of enzyme concentration and reaction time. The relationship between absorbance and condoliase concentration in the range of 0.01–5.0 U/ml (A). Condoliase in the concentration in the range of 0.01–0.6 U/ml was linearly correlated with absorbance (B). The relationship between absorbance and the incubation time with chondroitin sulfate. The concentration of condoliase is indicated by a line colored: red, 0.3 U/ml; orange, 0.15 U/ml; gray, 0.075 U/ml; blue, 0.03 U/ml (C). The absorbance of condoliase at concentration of 0.03 U/ml was linearly correlated with incubation time (D).

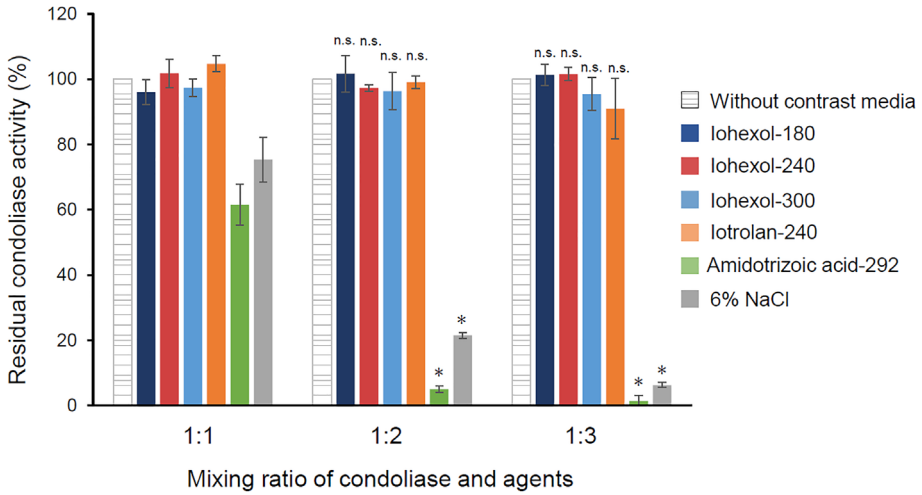

FIGURE 2 Condoliase activity exposed to different doses of contrast media or 6% NaCl. Condoliase solution (0.3 U/ml) was mixed with contrast media or 6% NaCl at ratios of 1:1, 1:2, and 1:3 in presence of CS. The absorbance of the sample without contrast media was set as 100%. Each measurement was performed three times, and the values represent the mean ± standard deviations. $^*p < 0.05$; n. s., not significant. Iotrolan and iohexol did not have significant effects on condoliase activity when mixed at both 1:2 and 1:3 ratio. Amidotrizoic acid-292 and 6% NaCl significantly and dose-dependently reduced condoliase activity.

absorbance at 585 nm: the coefficient of determination (r^2) was 0.998, and the absorbance reached a plateau when condoliase concentration exceeded 0.6 U/ml. Therefore, 0.3 U/ml was found to be the optimal condoliase concentration to evaluate the short-term influence of the contrast media on condoliase activity (incubation at 37°C for 10 min) in the presence of CS.

In order to determine how long the reaction time can be extended, we investigated the relationship between the reaction time

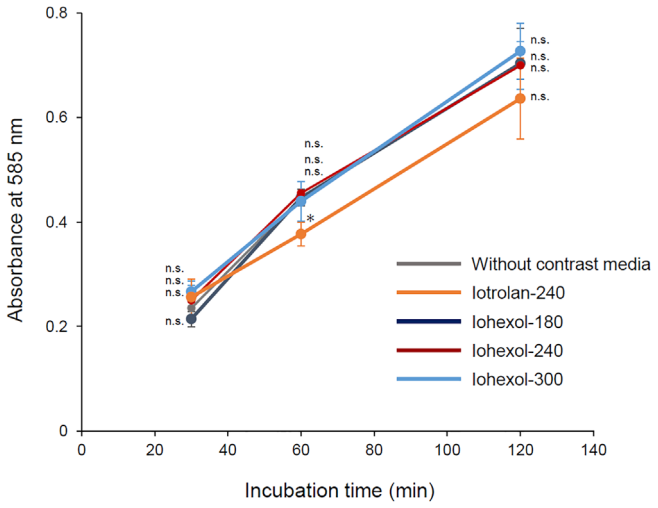

FIGURE 3 Condoliase activity when mixed with nonionic contrast media and chondroitin sulfate (CS). Condoliase (0.03 U/ml) was mixed with an equal volume of nonionic contrast media in the presence of CS, and the mixture was incubated at 37°C in the dark for 30, 60, and 120 min. Each measurement was performed 3 times, and the values represent the mean ± standard deviations ($n = 3–5$). *$p < 0.05$; n.s., not significant. There were no significant difference in condoliase activity between contrast media and control at all time points except for iotrolan-240 at 60 min (Dunnett's test for multiple comparison).

and absorbance at different condoliase concentrations (Figure 1C,D). At an enzyme concentration of 0.03 U/ml, absorbance had a linear relationship with reaction time; the coefficient of determination (r^2) was 0.986 up to 120 min. At an enzyme concentration of 0.075 U/ml or higher, the correlations between the absorbance and incubation time were not linear. Therefore, 0.03 U/ml was employed when a mixture of contrast media and condoliase was incubated in the presence of CS for 120 min at 37°C.

3.3 | Effects of contrast media on condoliase activity in presence of CS

As shown in Figure 2, the nonionic contrast media, iohexol-180, -240, and -300 and iotrolan-240, did not reduce the condoliase activity when incubated with CS at 37°C. Regardless of the volume of the contrast media added, even a three-fold volume, the residual condoliase activity was maintained above 90% ($p > 0.05$, Dunnett's test). On the other hand, the ionic contrast medium, amidotrizoic acid-292, reduced condoliase activity in a dose-dependent manner, and the activity almost disappeared when the volume of the contrast medium was doubled ($p < 0.05$, Dunnett's test). To evaluate the effect of hypertonic electrolytes on condoliase activity, we used 6% sodium chloride solution that has an osmotic pressure approximately six times that of normal saline and close to that of amidotrizoic acid. Similar to amidotrizoic acid-292, 6% NaCl reduced condoliase activity in a dose-dependent manner (Figure 2).

As shown in Figure 3, 90% of the activity of condoliase (0.03 U/ml) was maintained for at least 120 min, when mixed with an equal volume of all nonionic contrast media ($p > 0.05$, Dunnett's test). Although the reduction of condoliase activity by iotrolan 240 was significant at 60 min, significance disappeared at 120 min.

3.4 | Effects of contrast media on condoliase activity in absence of CS

When mixed with condoliase without adding CS, iohexol-180, -240, and -300 and iotrolan-240 reduced condoliase activity at room temperature in a time dependent manner (Figure 4). Condoliase activity in 0.9% NaCl (control) was maintained stably at its initial level for up to 60 min. Condoliase activity with iotrolan-240 was 65.2% at 60 min. Iohexol-180, -240 and -300 all reduced condoliase activity to the similar levels at all time points and the activity was approximately 35% at 60 min. A statistically significant difference in the reduction of enzyme activity was found between samples containing iohexol or iotrolan and the control at all time points ($p < 0.05$, Dunnett's test). Amidotrizoic acid-292 eliminated condoliase activity almost completely immediately after mixing. (Figure S3). Condoliase in 0.9% NaCl (control) was inactivated in 1 h when condoliase was stored in saline without CS at 37°C (Figure 5). Therefore, 24°C (room temperature) was employed to simulate the conditions of condoliase-contrast medium mixing and storage.

4 | DISCUSSION

In Japan, chemonucleolysis with condoliase has gradually been accepted as an alternative option for surgery in patients with LDH whose symptoms are unresponsive to conservative managements. Supported by the favorable results, a phase III clinical trial is ongoing in the United States.[18] The success of condoliase treatment depends on proper patient selection and its accurate injection into the NP. In some patients, discography is more accurate than MRI to establish the diagnosis of a subligamentous disc herniation, the best indication of condoliase, by depicting the patency of the posterior longitudinal ligament. Discography is also useful to determine whether the tip of the puncture needle is placed correctly inside the NP by assessing the dye pattern of the injected contrast medium. However, the concomitant use of contrast media with condoliase is currently prohibited because no data are available to judge if these two can be used together safely and effectively. The present study was conducted to test if contrast media have any effect on the activity of condoliase, the results of which would serve as a basis to pursue further studies to seek clinical application.

We, therefore evaluated the effect of two types, ionic and nonionic, of contrast media on condoliase activity under two conditions; presence or absence of CS, the former simulating that condoliase was intradiscally injected after discography, and later simulating that condoliase was mixed directly with a contrast medium prior to

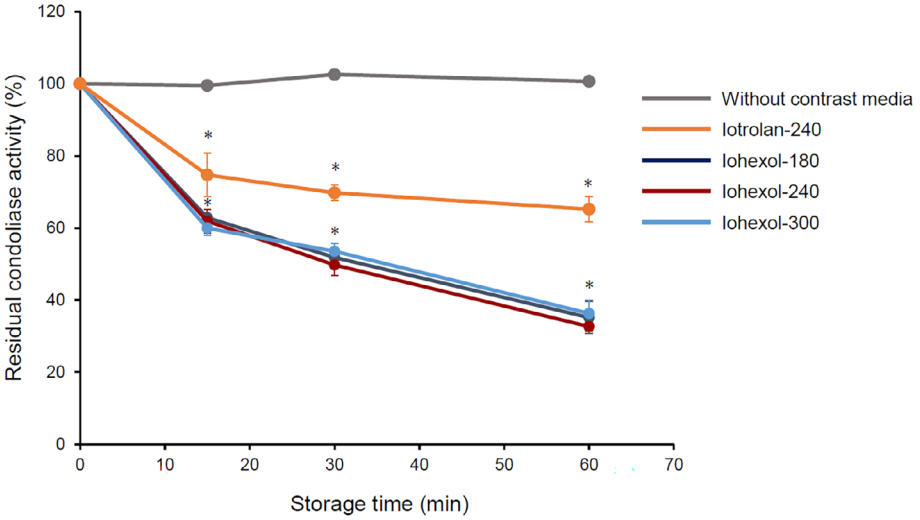

FIGURE 4 Condoliase activity when mixed with nonionic contrast media in the absence of chondroitin sulfate (CS). Condoliase solution (0.3 U/ml) was mixed with an equal volume of nonionic contrast media in the absence of CS, and the mixture was stored at room temperature in the dark for 0, 15, 30, and 60 min followed by measurement of condoliase activity by Morgan–Elson method. The condoliase activity in each sample was calculated based on the activity at 0 min of storage set as 100%. Each measurement was performed three times, and the values represent the mean ± standard deviations. *$p < 0.05$; a statistically significant difference in the reduction of enzyme activity was found between samples containing each iohexol or iotrolan and the control at all time points using Dunnett's test for multiple comparison.

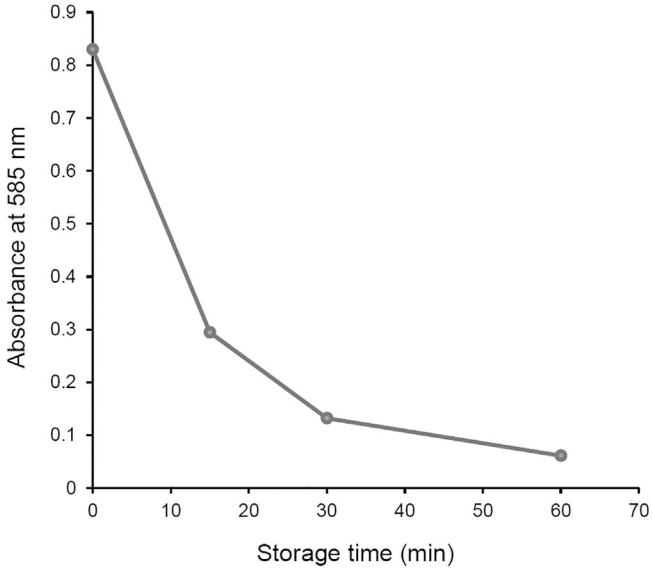

FIGURE 5 The activity of condoliase diluted in 0.9% saline without chondroitin sulfate (CS) was stored for 60 min in the dark at 37°C. Saline (0.9% NaCl) was added to make the final condoliase concentration 0.3 U/ml. The condoliase solution was stored at 37°C in the dark for 0, 15, 30, and 60 min followed by measurement of condoliase activity by Morgan–Elson method. Condoliase was inactivated within 1 h when stored in saline without CS at 37°C.

discography. The results obtained in this study are summarized in Table 2. In the presence of CS, ionic contrast media reduced condoliase activity, but nonionic contrast media did not. However, in the absence of CS, the ionic contrast medium completely eliminated condoliase activity and the nonionic media also reduced condoliase activity in a time-dependent manner. Hence, condoliase loses its enzyme activity when mixed directly with any kind of iodinated contrast medium.

Amidotrizoic acid-292, which is a high-osmolar agent containing ionic monomers, reduced condoliase activity in a dose-dependent manner in the presence or absence of CS. Generally, salt is one of the major substances that affect protein solubility. At a low salt concentration, protein solubility increases as the salt concentration rises, a phenomenon termed "salting in." Meanwhile, at a high salt concentration, the solubility of the proteins drops sharply and proteins precipitate, a process termed "salting out." When salt concentration is increased, the water molecules are attracted by the salt ions, and protein–protein interactions become stronger via hydrophobic interaction or van der Waals interaction of protein molecules leading to the self-aggregation of protein. The inactivation of condoliase by a hypertonic solution such as ionic contrast media may be due to such protein aggregation.

Although nonionic contrast media did not reduce condoliase activity (Figure 2) when mixed with CS, this could be attributable to the short interaction time of only 10 min. We, therefore, evaluated if condoliase activity would be affected by longer incubation times with contrast media. As shown in Figure 1C, the absorbance reached a plateau beyond a certain time points when the concentrations of enzyme were high because the enzyme might have exhausted the substrate, CS. Therefore, we needed to detect an enzyme concentration that would maintain a linear correlation between the incubation time and condoliase activity for at least a few hours. Results of our preliminary experiment suggested that 0.03 U/ml is the appropriate concentration of condoliase that allows to extend the incubation time of condoliase with contrast agents up to 2 h in the presence of CS (Figure 1C,D).

TABLE 2 Summary of effects of contrast media on condoliase activity

Classification	Active ingredient (mg/ml)	Condoliase activity	
		CS present[a]	CS absent[b]
Ionic type	Amidotrizoic acid (471.8 mg/ml)	Decreased	Decreased
Nonionic type	Iohexol (388.2 mg/ml)	Not affected	Decreased
	Iohexol (517.7 mg/ml)	Not affected	Decreased
	Iohexol (647.1 mg/ml)	Not affected	Decreased
	Iotrolan (512.6 mg/ml)	Not affected	Decreased

[a]The mixtures of condoliase and contrast media with CS were incubated at 37°C for 10 min. The absorbance of condoliase solution without contrast media was set as 100%.
[b]The mixtures of condoliase and contrast media without CS were stored at room temperature for 15–60 min. The absorbance of each sample measured at 0 min (no storage) was set as 100%.
Abbreviation: CS, chondroitin sulfate.

Using this condition, we found that nonionic contrast media did not affect condoliase activity even if the incubation time was extended to 2 h (Figure 3).

Generally, enzyme stability in a solution is influenced by the presence of the substrate. Accordingly, condoliase activity was maintained in the presence of CS at 37°C for at least 2 h. It was therefore suspected that the direct effect of contrast media on condoliase cannot be evaluated in the presence of CS (Figures 2 and 3). To evaluate the direct effect of contrast media on condoliase, we, therefore, measured condoliase activity when mixed with contrast media without CS (Figure 4). However, when condoliase was stored in saline without CS at 37°C, condoliase activity was lost rapidly within 1 h (Figure 5); hence, in order to directly assess the effect of contrast media on condoliase, the storage temperature had to be optimized so that condoliase would not lose its activity. As shown in Figure 4, when stored at room temperature (24°C), condoliase in saline maintained its activity stably for at least 60 min without CS. Using this condition, we found that nonionic contrast media reduced condoliase activity in a time-dependent manner (Figure 4).

Iohexol-180, -240 and -300 have different iodine concentrations, and characteristic physical properties such as viscosity (Table 1). However, there were no significant difference in condoliase activity among three different concentrations of iohexol. Iodine concentration and the physical properties of contrast media seem to have no effects on condoliase activity. In the experiments shown in Figure 4, the absorbances of solutions containing iohexol and iotrolan measured at time 0 were 0.48 and 0.49, respectively, whereas that of control was 0.41; therefore, neither iotrolan nor iohexol affected condoliase activity at the start of storage. Although iotrolan, when compared to iohexol, showed less reduction in condoliase activity, the reason is unclear. Differences in factors other than the active ingredient, such as the type and composition of additives, might have affected condoliase activity. Additional research is required to identify such other influential factors.

The present in vitro study has several limitations:

(i) Condoliase activity may be maintained by the CS abundant in the nucleus pulposus; however, the safety and stability of condoliase when administered to humans after contrast media injection have not been tested and are unknown.

(ii) The exact cause of the reduction in condoliase activity by contrast media is unknown.

This is the first study to evaluate the effects of contrast media on condoliase activity in vitro, and further in vivo studies are necessary to verify the clinical relevance of the present study.

5 | CONCLUSION

Condoliase activity was significantly reduced by ionic type contrast media. Although the activity of condoliase was not affected by nonionic contrast media in the present of CS, it was reduced in the absence of CS. Therefore, any contrast media should not be mixed directly with condoliase.

AUTHOR CONTRIBUTIONS

Ippei Watanabe and Taiichi Shirogane conceived and designed the experiments, and Ippei Watanabe performed the experiments. Ippei Watanabe and Taiichi Shirogane wrote the manuscript. Yukihiro Matsuyama and Kazuhiro Chiba edited the manuscript from clinical and scientific standpoints. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors are grateful to Ken Tochiyama, Takayuki Seo and Kazuhiro Kojima (Seikagaku Corp.) for concerning the statistical analyses. We thank Ayako Katsuyama (Seikagaku Corp.) and Tadamasa Kobayashi (Seikagaku Corp.) for helpful discussion. We also thank Kiyoshi Suzuki (Seikagaku Corp.) and Hideyuki Futatsumori (Seikagaku Corp.) for skillful technical support in the handling of the enzyme.

CONFLICT OF INTEREST

Ippei Watanabe and Taiichi Shirogane are employees of Seikagaku Corp. This study was conducted at Seikagaku Corp. Yukihiro Matsuyama and Kazuhiro Chiba received consulting fee from Seikagaku Corp.

ORCID

Kazuhiro Chiba https://orcid.org/0000-0002-4078-5258

REFERENCES

1. Truumees E. A history of lumbar disc herniation from Hippocrates to the 1990s. *Clin Orthop Relat Res*. 2015;*473*(6):1885-1895.
2. Deyo RA, Mirza SK. Clinical practice. Herniated lumbar intervertebral disk. *N Engl J Med*. 2016;*374*(18):1763-1772.
3. Smith L, Garvin PJ, Gesler RM, et al. Enzyme dissolution of the nucleus pulposus. *Nature*. 1963;*29*(198):1311-1312.
4. Smith L. Enzyme dissolution of the nucleus pulposus in humans. *JAMA*. 1964;*11*(187):137-140.
5. Wardlaw D, Rithchie IK, Sabboubeh AF, Vavdha M, Eastmond CJ. Prospective randomized trial of chemonucleolysis compared with surgery for soft disc herniation with 1-year, intermediate, and long-term outcome: part I: the clinical outcome. *Spine (Phila Pa 1976)*. 2013;*38*(17):E1051-E1057.
6. Nordby EJ, Wright PH. Efficacy of chymopapain in chemonucleolysis. A review. *Spine (Phila Pa 1976)*. 1994;*19*(22):2578-2583.
7. Javid MJ. A 1- to 4-year follow-up review of treatment of sciatica using chemonucleolysis or laminectomy. *J Neurosurg*. 1992;*76*(2):184-190.
8. Matsuyama Y, Chiba K. Condoliase for treatment of lumbar disk herniation. *Drugs Today (Barc)*. 2019;*55*(1):17-23.
9. Agre K, Wilson RR, Brim M, et al. Chymodiactin postmarketing surveillance. Demographic and adverse experience data in 29,075 patients. *Spine (Phila Pa 1976)*. 1984;*9*(5):479-485.
10. Yamagata T, Saito H, Habuchi O, Suzuki S. Purification and properties of bacterial chondroitinases and chondrosulfatases. *J Biol Chem*. 1968;*243*(7):1523-1535.
11. Hamai A, Hashimoto N, Mochizuki H, et al. Two distinct chondroitin sulfate ABC lyases. An endoeliminase yielding tetrasaccharides and an exoeliminase preferentially acting on oligosaccharides. *J Biol Chem*. 1997;*272*(14):9123-9130.
12. Olczyk K. Age-related changes in glycosaminoglycans of human intervertebral discs. *Folia Histochem Cytobiol*. 1993;*31*(4):215-220.
13. Takashima M, Watanabe I, Miyanaga A, Eguchi T. Substrate specificity of chondroitinase ABC I based on analyses of biochemical reactions and crystal structures in complex with disaccharides. *Glycobiology*. 2021;*31*(11):1571-1581.
14. Iwata H, Matsuyama Y, Kato F, et al. Chemonucleolysis with condoliase for lumbar disc herniation. *Orthop Surg Traumatol*. 2019;*62*:269-282.
15. Chiba K, Matsuyama Y, Seo T, Toyama Y. Condoliase for the treatment of lumbar disc herniation: a randomized controlled trial. *Spine (Phila Pa 1976)*. 2018;*43*(15):E869-E876.
16. Matsuyama Y, Chiba K, Iwata H, Seo T, Toyama Y. A multicenter, randomized, double-blind, dose-finding study of condoliase in patients with lumbar disc herniation. *J Neurosurg Spine*. 2018;*28*(5):499-511.
17. Chiba K, Matsuyama Y, Kato F, et al. Potency and limitation of condoliase treatment for lumbar disc herniation based on evidence obtained in basic researches—a 50-years history from initial concept to clinical approval. *J Jpn Orthop Assoc*. 2021;*95*:1144-1160.
18. U.S. National Library of Medicine. ClinicalTrials.gov: SI-6603 (Condoliase) Study for Lumbar Disc Herniation (Discovery 6603 Study). Accessed June 6, 2022. https://clinicaltrials.gov/ct2/show/NCT03607838?term=condoliase&draw=2&rank=3
19. Takeda T, Narimatsu Y, Hiramatsu K. Fukubukekkanzoueiniokeru zoueizai. *J Jpn Soc Biorheol*. 1995;*9*:23-28. (in Japanese).
20. Widmark JM. Imaging-related medications: a class overview. *Proc (Bayl Univ Med Cent)*. 2007;*20*(4):408-417.
21. Mori M, Hanakita J, Takahashi T, Minami M, Honda F, Arita K. Analysis of the usefulness of lumbar discography in the MRI era. *Spine Surg*. 2010;*24*(2):203-207. (in Japanese).
22. Husted SE, Kanstrup H. Thrombotic complications in coronary angioplasty—ionic versus non-ionic low-osmolar contrast media. *Acta Radiol*. 1998;*39*(4):340-343.
23. Resshing JL, Strominger JL, Leloir LF. A modified colorimetric method for the estimation of *N*-acetylamino sugars. *J Biol Chem*. 1955;*217*(2):959-966.
24. Prabhakar V, Raman R, Capila I, Bosques CJ, Pojasek K, Sasisekharan R. Biochemical characterization of the chondroitinase ABC I active site. *Biochem J*. 2005;*390*(Pt 2):395-405.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Watanabe, I., Shirogane, T., Matsuyama, Y., & Chiba, K. (2022). Effect of contrast media on the enzyme activity of condoliase: In vitro assessment. *JOR Spine*, *5*(3), e1221. https://doi.org/10.1002/jsp2.1221