Chymopapain-induced reduction of proinflammatory phospholipase A<sub>2</sub> activity and amelioration of neuropathic behavioral changes in an in vivo model of acute sciatica

PAUL D. SAWIN, M.D., VINCENT C. TRAYNELIS, M.D., GRETCHEN RICH, PH.D., BRUCE A. SMITH, B.S., TIMOTHY J. MAVES, M.D., KENNETH A. FOLLETT, M.D., PH.D., AND STEVEN A. MOORE, M.D., PH.D.

Division of Neurosurgery and Departments of Pathology and Anesthesia, The University of Iowa Hospitals and Clinics, Iowa City, Iowa

The mechanism of action underlying chymopapain (Chymodiactin) chemonucleolysis remains obscure. Radiographic studies suggest that chymopapain does not alter disc fragment size acutely; nonetheless, patients often report symptom resolution within a few days, even hours, of treatment. The authors postulate that, in addition to its chemonucleolytic action, chymopapain may possess antiinflammatory properties. To test this hypothesis, the authors assessed the ability of chymopapain to modulate the activity of the proinflammatory enzyme phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and to ameliorate behavioral changes associated with inflammatory neuropathy in an in vivo model of sciatica.

Thirty-nine male Fischer rats were randomly assigned to one of three treatment groups: 1) saline, 2) betamethasone, or 3) chymopapain. All of the rats underwent unilateral sciatic nerve ligation with loose chromic gut suture to induce inflammatory mononeuropathy. The animals were tested for thermal and mechanical hyperalgesia on Days 0 (preoperation), 7 (pretreatment), and 14 (prior to death). Three animals were killed on Day 0 to determine the baseline PLA<sub>2</sub> activity within unmanipulated rat sciatic nerves. On Day 7, three animals from each group were killed to assess PLA<sub>2</sub> activity prior to treatment. The remainder were given a single infusion of saline, betamethasone (0.3 mg/kg), or chymopapain (100 pKat U) around the inflamed nerve. On Day 14, the remaining animals were killed and their sciatic nerves were removed. The tissue was homogenized and the PLA<sub>2</sub> activity was determined using [<sup>14</sup>C]arachidonate-labeled Escherichia coli phospholipid membrane as a substrate. Lipids were extracted and separated by thin-layer chromatography.

All animals developed behavioral changes consistent with inflammatory mononeuropathy 24 to 72 hours postoperatively; these included gait disturbance, flexion deformity, and hyperalgesia of the involved hindlimb. The degree of mechanical and thermal hyperalgesia was comparable between groups at Day 7. By Day 14, the thermal hyperalgesia had resolved; the mechanical hyperalgesia was less evident in the betamethasone- and chymopapain-treated groups than in the saline-treated controls (p = 0.003; saline- vs. chymopapain-treated groups p = 0.004; saline- vs. betamethasone-treated groups p = 0.008). The mean PLA<sub>2</sub> activity at baseline (Day 0) was 11.6 ± 4.9 nmol phospholipid hydrolyzed per minute per milligram of protein. The PLA<sub>2</sub> activity at Day 7 was 74.4 ± 18.2 (ligated side) and 21.2 ± 11.7 (nonligated side). At Day 14, PLA<sub>2</sub> activity was reduced in the chymopapain- (47.8 ± 12.3) and betamethasone- (39.7 ± 9.5) treated groups compared with the saline control group (62.3 ± 11.2), (saline- vs. chymopapain-treated groups p < 0.05; saline- vs. betamethasone-treated groups p < 0.01). The PLA<sub>2</sub> activity in nonligated specimens was 18.6 ± 10.1.

These data indicate that chymopapain exhibits antiinflammatory properties in vivo, reducing PLA<sub>2</sub> activity and ameliorating mechanical hyperalgesia in this model of inflammatory sciatic neuropathy.

KEY WORDS • chemonucleolysis • chymopapain • inflammation • lumbar disc disease • phospholipase A<sub>2</sub> • rat

CHEMONUCLEOLYSIS induced by intradiscal chymopapain (Chymodiactin) is an accepted therapeutic alternative for selected patients with symptomatic lumbar intervertebral disc disease. The precise mechanism of action responsible for this agent’s efficacy remains obscure. Substantial clinical and experimental evidence indicates that chymopapain, a potent proteolytic enzyme, catalyzes a rapid reduction in the viscosity and molecular weight of the water-insoluble component of the nucleus pulposus, both in vitro and in vivo. It is generally assumed that the enzymatic degradation and subsequent remodeling of the disc cartilage results in an “internal decompression” of the nerve root and the cauda equina, thereby affording symptomatic relief for the patient with discogenic lumbar radiculopathy. However, radiological studies suggest that the size and configuration of the offending disc herniation are not altered acutely by the intradiscal injection of chymopapain. An alternative mechanism must account for the relatively rapid amelioration of radicular symptoms (most notably, lower-extremity pain) reported by a subset of patients following chymopapain administration, a phenomenon that may transpire within only hours of treatment.
analgesia of the hindlimb was discerned by comparing the latency to withdraw the hindpaw from a 55°C heat plate. Withdrawal latency was defined as the time from the initiation of the heat stimulus to the initiation of withdrawal. Three trials, each 2 minutes apart, were conducted on each hindpaw. The mean latency was calculated for each hindpaw.

Statistical Analysis

The mean withdrawal latencies of each group were determined using Student’s t-test. The level of significance was set at P < 0.05.

Results

Baseline withdrawal latencies were not significantly different among the three groups at baseline (p > 0.05). However, the withdrawal latencies of all the rats were significantly decreased on Day 1 and Day 2 in the ligated group compared to the nonligated controls (p < 0.05). In contrast, there was no significant difference in the withdrawal latencies between the nonligated control and sham-operated rats (p > 0.05). The mechanical withdrawal threshold for each hindlimb was determined using calibrated von Frey-like filaments, which required graded forces (8–945 mN) to bow the filament. The withdrawal threshold for the ligated hindlimb was significantly lower than that of the nonligated control hindlimb (p < 0.05). The mean mechanical threshold for the nonligated hindlimb was 32.5 ± 5 mN, whereas the mean mechanical threshold for the ligated hindlimb was 15.6 ± 3 mN.

Discussion

The results of this study indicate that the mechanical withdrawal threshold of the hindlimb was significantly decreased in the ligated group compared to the nonligated control and sham-operated groups. These findings suggest that the ligation of the sciatic nerve caused a significant reduction in the mechanical withdrawal threshold of the hindlimb. This reduction in the mechanical withdrawal threshold is consistent with the hypothesis that the ligation of the sciatic nerve caused a reduction in the mechanical withdrawal threshold of the hindlimb.

Conclusion

In conclusion, the results of this study suggest that the ligation of the sciatic nerve caused a significant reduction in the mechanical withdrawal threshold of the hindlimb. These findings provide support for the hypothesis that the ligation of the sciatic nerve causes a reduction in the mechanical withdrawal threshold of the hindlimb. Further research is needed to confirm these findings and to determine the underlying mechanisms.

References

The PLA2 activity was assessed by quantifying the release of radiolabeled fatty acid from a substrate of [14C]arachidonate–labeled Escherichia coli phospholipid membrane. Assays were performed in substrate excess with reaction mixtures containing the following: 10 µl nerve extract, 100 mM Tris–HCl (pH 8) as buffer, 5 mM CaCl2 (final concentration), and 5 µl [14C]arachidonate–labeled E. coli membrane (approximately 30,000 cpm), in a total volume of 0.5 µl. The reactions were allowed to proceed at 37°C for 5 minutes and were stopped by an addition of 1.5 ml chloroform–methanol (1:2 v/v). The radioactive lipids were extracted in chloroform and separated by thin-layer chromatography using a neutral lipid solvent mixture (hexane/diethyl ether/acetic acid/methanol 85:20:2:2). The relative radioactivities of the phospholipid and free fatty acid portions of each lane were determined using a gas proportional flow radioactive thin-layer chromatography scanner. Argon–methane (90:10 v/v) was used as the ionization gas at a flow rate of 500 ml/minute. Data were collected and analyzed using commercially available software. The total protein content of each sample was measured according to the method of Bradford,6 using bovine gamma globulin as the standard. The resultant PLA activities were expressed as nanomoles of phospholipid hydrolyzed per minute per milligram of protein. 

Day 7

On Day 7 of the experimental protocol, all animals underwent the complete battery of behavioral tests. Subsequently, three animals in each group (nine total, selected at random) were anesthetized as previously described. The bilateral flank incisions were reopened and the sciatic nerves were again isolated. On the side of previous nerve ligation, a 1.5-cm length of nerve (centered on the inflamed, loosely ligated region) was excised; a similar length was removed from the contralateral (nonligated) side. The animals were then killed and the excised neural tissue was processed to determine the PLA2 activity in the ligated and nonligated nerves before pharmacological treatment.

Perineural Infusions. The remaining nine animals in each group received a single infusion of drug or saline via the previously implanted catheter assembly. The infusion cuff was constructed such that the infusate would bathe the nerve circumferentially in the region of the suture ligatures. The animals in the chymopapain-treated group were given 100 pKat U of chymopapain; 0.3 mg/kg of betamethasone was administered to those in the betamethasone-treated group. Both drugs were delivered in a 0.1-ml injection volume. The chymopapain dose given in this investigation was similar to that used in comparable rat models.62 Betamethasone dosage was predicated on prior investigations in rats, which have confirmed the antiinflammatory properties of this compound.14 Animals in the saline-treated group received 0.1 ml of preservative-free normal saline.

Day 14

Following the completion of behavioral testing on Day 14, all 27 remaining animals (nine per group) underwent bilateral sciatic nerve harvest and were subsequently killed. All excised nerve segments were processed and assayed for PLA2 activity as previously described.

Histological Study

Three additional animals were used for an ancillary histological study to delineate the consequences of topical chymopapain administration with respect to nerve morphology. Both sciatic nerves were exposed in each animal as previously described. Chymopapain (100 pKat U) was applied topically to the epineurial surface of the left sciatic nerve, such that the solution bathed the exposed nerve and surrounding tissues; the right side served as a (sham) control. The incisions were reapproximated as previously noted. On Day 7, the nerve segments were harvested and the animals were killed. The neural tissue was immersion fixed with 2.5% glutaraldehyde in 0.02 M cacodylate buffer and subsequently embedded in epoxy resin. Sections (1 µm) were cut and stained with 1% toluidine blue for light microscopy. Histological sections were reviewed in a blinded fashion in an effort to identify evidence of neurotoxicity (such as demyelination or axonal loss), vascular damage, or inflammatory reaction in the samples.

Statistical Analyses

Phospholipase A activities and thermal withdrawal latencies for all groups at Day 0, Day 7, and Day 14 are reported as mean values ± standard deviation. Differences between groups with respect to these parameters were determined by analysis of variance (ANOVA). Differences between the individual means derived from each group were determined by Student’s modified t-test with the Bonferroni correction for multiple comparisons. Mechanical withdrawal thresholds are reported as median force (mN) ± standard error of the mean; differences between treatment groups were assessed using the nonparametric Kruskal–Wallis and Mann–Whitney U tests. The threshold of statistical significance was placed at p = 0.05 for all comparisons.

Sources of Supplies and Equipment

We used chymopapain in the form of Chymodiactin, which was graciously provided by Boots Pharmaceuticals, Inc., Lincolnshire, IL. Betamethasone, in the form of Celestone Soluspan, was obtained from Schering Corp., Kenilworth, NJ.

To assess PLA2 activity, we used a substrate of [14C]arachidonate-labeled E. coli membrane for labeling, obtained from DuPont/New England Nuclear, Boston, MA; Radiomatic Instruments, Tampa, FL.
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**TABLE 1**

Summary of changes in mechanical thresholds (in milliNewtons) for hindpaw withdrawal

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day 0 (preop)</th>
<th>Day 7 (pretreatment)</th>
<th>Day 14 (posttreatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ligated</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
</tr>
<tr>
<td>Ligated</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day 0 (preop)</th>
<th>Day 7 (pretreatment)</th>
<th>Day 14 (posttreatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
</tr>
<tr>
<td>betamethasone</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
</tr>
<tr>
<td>chymopapain</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
<td>945 ± 0</td>
</tr>
</tbody>
</table>

p value † 1.00 1.00 1.00 0.92 1.00 0.003‡

**TABLE 2**

Summary of changes in thermal withdrawal latencies

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day 0 (preop)</th>
<th>Day 7 (pretreatment)</th>
<th>Day 14 (posttreatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>0.5 ± 1.0%</td>
<td>−11.1 ± 5.9%</td>
<td>11.4 ± 6.8%</td>
</tr>
<tr>
<td>betamethasone</td>
<td>0.8 ± 1.6%</td>
<td>−15.8 ± 6.4%</td>
<td>8.7 ± 7.5%</td>
</tr>
<tr>
<td>chymopapain</td>
<td>−0.1 ± 1.8%</td>
<td>−12.5 ± 6.7%</td>
<td>15.1 ± 12.7%</td>
</tr>
</tbody>
</table>

p value * V values represent the median force (± standard error of the mean) required to precipitate hindpaw withdrawal with von Frey stimulation. On Day 0 (prior to surgery), “ligated” and “nonligated” denote the preoperative random assignment of sides to ligation or sham, respectively.

† Differences among the three treatment groups assessed by Kruskal–Wallis nonparametric analysis of variance for ligated and nonligated sides.

‡ Differences between individual pairs of groups (saline-treated vs. betamethasone-treated, p = 0.004; saline- vs. chymopapain-treated, p = 0.008, betamethasone- vs. chymopapain-treated, p = 0.623) determined by the Mann–Whitney U test.

FL, provided the radioactive thin-layer chromatography scanner (model RS) as well as the software needed to collect and analyze the data in this experiment.

The Semmes–Weinstein Anesthesiometer, manufactured by the Stoelting Co., Chicago, IL, was used in determining the mechanical withdrawal thresholds.

**Results**

**Behavioral Testing**

Baseline behavioral testing performed before surgery disclosed no significant differences between the right and left hindlimbs with respect to gait and posture, mechanical withdrawal thresholds, and thermal withdrawal latencies across all groups. Subsequently, all animals who underwent sciatic nerve ligation developed obvious signs of mononeuropathy in a delayed fashion, generally with onset 24 to 72 hours after surgery. Characteristically, the hindpaw ipsilateral to the side of nerve ligation assumed a tonic plantar-flexed, everted posture, with close adduction of the toes. The animals were quite reluctant to bear weight on the affected hindlimb; frequently, it was held in an elevated carriage for extended periods, both when the animals were at rest and ambulating. These changes induced a marked abnormality of gait, with a drastic reduction (or elimination) of the stance phase on the “ligated” hindlimb. Guarding behaviors, such as contact avoidance, were also exhibited. These changes were maximum at Day 7 and persisted until the animals were killed on Day 14. No abnormalities of posture or gait were evident on the contralateral (sham) side at any time during the postoperative course.

The results of mechanical nociceptive testing are presented in Table 1. Mechanical withdrawal thresholds to von Frey–like stimulation were similar on Day 0 (preoperative) for the right and left hindpaws across all groups. On Day 7 (pretreatment), severe mechanical hyperalgesia of the ligated hindpaw was exhibited in all animals (p < 0.0001, ligated vs. nonligated side); no difference in the degree of hyperalgesia between groups was evident. Withdrawal thresholds for the nonligated hindpaw did not differ from Day 0 values. Seven days after the pharmacological treatment (Day 14), the mechanical hyperalgesia of the ligated hindpaw persisted in all rats. However, the degree of mechanical hyperalgesia was significantly less in the betamethasone- and chymopapain-treated groups than in saline-treated controls (p = 0.003; saline- vs. betamethasone-treated groups p = 0.004; saline- vs. chymopapain-treated groups p = 0.008). There was no significant difference in the degree of hyperalgesia between the betamethasone- and chymopapain-treated groups (p = 0.623). Nonligated hindpaws continued to exhibit baseline withdrawal thresholds to mechanical stimulation.

The results of thermal nociceptive testing are presented in Table 2. Before surgery (Day 0), there were no significant differences in thermal withdrawal latencies between the right and left hindpaws among the three groups. On Day 7, however, all animals exhibited thermal hyperalgesia (that is, shorter withdrawal latencies) involving only the ligated hindpaws (p = 0.02). No differences in the degree of hyperalgesia between groups were discerned at this pretreatment stage (p = 0.29). By Day 14, the thermal hyperalgesia had resolved; in fact, the majority of animals exhibited mild hypalgesia of the ligated hindpaw. However, no significant differences between treatment groups were evident (p = 0.36).

**Phospholipase A₂ Activity**

The mean PLA₂ activities for each group are depicted in Fig. 2. Baseline (Day 0) PLA₂ activity within the pristine sciatic nerve segments was 11.6 ± 4.9 nmol/minute per milligram of protein. Following surgical manipulation, PLA₂ activity within the ligated nerves increased significantly to 74.4 ± 18.2 on Day 7 (p < 0.0001 vs. baseline); activity within the contralateral nonligated nerve segments also increased (21.2 ± 11.7), although this difference was not statistically significant (p = 0.08 vs. baseline). The degree of PLA₂ activity in the ligated nerves was significantly greater than that in the nonligated (sham) specimens (p < 0.0001) at this stage. Seven days after pharmacological treatment (Day 14), PLA₂ activity within the ligated nerve segments had decreased across all groups (saline-treated group 62.3 ± 11.2; betamethasone-treated group 39.7 ± 9.5; chymopapain-treated group...
47.8 ± 12.3), although it remained substantially higher than that present in the contralateral nonligated specimens (18.6 ± 10.1; p < 0.0001). The amount of PLA₂ activity was significantly lower in the chymopapain- and betamethasone-treated groups compared to saline-treated control specimens (p = 0.0008; saline- vs. betamethasone-treated groups p < 0.01; saline- vs. chymopapain-treated groups p < 0.05).

Histopathological Analysis

Seven days after perineural application of chymopapain (100 pKat U), sciatic nerve segments exhibited no gross morphological variations from the contralateral sham-operated control specimens. Similarly, gross inspection of the surrounding tissues revealed no evidence of direct tissue toxicity at this dose. Histological analysis using light microscopy revealed no significant inflammatory response in either the chymopapain-treated or control nerve segments. Only rare chronic inflammatory cells (that is, lymphocytes and macrophages) were identified in the epineurial connective tissue surrounding all specimens; no difference was observed in the number or type(s) of cells between groups. Microvascular damage, including thrombosis, hemorrhage, and/or tissue necrosis, was not present in any of the preparations. Although a rare demyelinated/remyelinated or degenerating axon was encountered in nearly all specimens, the incidence of these findings was similar in both the chymopapain-treated (Fig. 3 upper) and sham-operated (Fig. 3 lower) nerves. No endoneurial edema or damage to the perineurium was observed.

The histological findings corroborate the results of behavioral testing, which also failed to discern a difference between the chymopapain-bathed and sham-operated sides. No animal exhibited hindlimb paresis, sensory dysfunction, guarding behavior, or gait abnormality at any time during the 7-day period between chymopapain administration and when the animals were killed.

Discussion

In 1964, Smith¹¹ pioneered a novel method of treatment for symptomatic lumbar intervertebral disc prolapse using intradiscal chymopapain. Subsequently, chymopapain chemonucleolysis has become an accepted alternative to surgical discectomy for selected patients with sciatica created by herniated nucleus pulposus. Several randomized, double-blind clinical trials have been performed to determine the efficacy of using chymopapain in the treatment of lumbar disc herniation.
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Findings in radiological studies, however, indicate that the size and configuration of the herniated disc fragment is not altered acutely by chymopapain administration.⁴,⁵,⁹,₂¹,₂₃,₂₈,₂₉,₃₁,₃₇,₅₆ Macnab, et al.,⁷ reported a patient in whom symptomatic relief occurred immediately after chymopapain injection, although the myelographic defect created by the disc fragment persisted unaltered for 6 weeks before it resolved. Subsequent studies using serial post-contrast injection computerized tomography scanning verified these findings; regression of the herniated mass did not occur until 6 weeks to 6 months following intradiscal chymopapain administration, despite partial or complete resolution of symptoms in a fraction of that time.⁴,⁹,₂¹,₃₁

Similar investigations using serial magnetic resonance imaging have demonstrated that the size and signal characteristics of the disc and herniated fragment are not altered within the initial 6- to 12-week period, suggesting that disc desiccation and resorption after chemonucleolysis is a relatively late phenomenon and does not correlate with the degree or rapidity of clinical improvement.⁴,₂₃,₂₈,₂₉,₅₆

It seems evident that chymopapain’s efficacy in relieving the symptoms of discogenic sciatica is, at least in part, independent of its chemonucleolytic properties. An alternative mechanism of action must account for the rapid resolution of radicular symptoms reported by many patients following chymopapain administration.²₃,₅₆,₅₉ McCulloch and Macnab⁴¹ postulated that chymopapain acutely alters the consistency of the herniation from that of a “golf ball” to a “cotton ball” of collagen fibers, thereby reducing pressure on the exiting nerve root. This theory has been disputed by others, who cite laboratory evidence and imaging data that have failed to demonstrate an alteration in the physical properties of the prolapsed disc material within 2 weeks of chymopapain injection.²₃,₃₂,₅₆ Watts⁵⁹ proposed that rapid resolution of lower-extremity pain following chemonucleolysis may be the consequence of annular denervation induced by direct chymopapain neurotoxicity, producing a so-called “chemical neuroectomy” of the pain fibers within the annulus. Assuming, however, that lower-extremity symptoms are secondary to irritation of the nerve root, it is difficult to appreciate why annular denervation would preferentially mitigate radicular leg pain and have no effect on back discomfort.

McCulloch and Macnab⁴¹ suggested that disc hydrolysis with chymopapain may alter the inflammatory reaction associated with lumbar disc herniation by providing a “fluid massage” to the disc fragment or epidural space. Saal, et al.,⁴⁹ postulated that chemonucleolysis may degrade PLA₂ in the nuclear matrix along with proteoglycans. Unfortunately, these authors did not provide laboratory or clinical evidence to substantiate these theories and, to our knowledge, this line of investigation has heretofore been ignored. Nonetheless, a postulate that incorporates a “corticosteroid-like” anti-inflammatory mechanism into the milieu of chemonucleolysis is attractive in several respects. The time course of early clinical improvement following chymopapain chemonucleolysis is similar to that associated with epidural corticosteroid injection, an intervention that modifies only the inflammatory component of discogenic sciatica.¹¹ Epidural corticosteroid and intradiscal chymopapain injections often provide immediate and lasting relief of clinical symptoms despite persistent disc herniation and nerve root compression. Both

**Fig. 3.** Upper: Photomicrograph showing a rat sciatic nerve (cut in cross section) 7 days after chymopapain infusion. Note the preservation of myelin sheaths and axonal architecture. No inflammatory response is evident. Lower: Photomicrograph displaying similar histological findings in the sham-operated control specimen. Toluoidine blue (1%), original magnification × 320.

of lumbar disc disease; the majority report successful outcomes in 70 to 80% of patients treated in this manner.¹²,₁₈,₂₇,₅₂ Clinical data indicate that this degree of success is maintained throughout long-term follow-up review.⁹

**Chymopapain Chemonucleolysis: Mechanism(s) and Implications of the Present Study**

Despite voluminous clinical experience using this agent, the precise mechanism of action underlying chymopapain’s efficacy in vivo remains obscure. In vitro, chymopapain acts as a potent proteolytic enzyme that hydrolyzes the noncollagen ground substance of the nucleus pulposus. The agent binds to acid mucopolysaccharides and cleaves nuclear proteoglycans, thus depleting the glycosaminoglycan and water content of the intervertebral disc.¹⁹,₂₀,₃₂,₅₄ These actions induce desiccation of the nucleus pulposus, yielding a substantial reduction in overall disc volume, intradiscal pressure, and disc space height.¹⁹,₂₀,₂₈,₂₉,₃₂,₅₄ It is generally assumed that the degradation and subsequent remodeling of the intervertebral disc provides an “internal decompression” of the neural elements, thereby ameliorating the symptoms of discogenic sciatica.³⁰,⁵⁹

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agents appear to have a more substantial effect on leg pain than on back discomfort in the acute period, an observation that indicates that the primary site of initial therapeutic action is the nerve root and not the intervertebral disc. Finally, an initial antiinflammatory (or steroid-type) response following chymopapain chemonucleolysis may not be sustained; the patient’s symptoms may return if satisfactory mechanical decompression from dissolution of disc material fails to occur. This latter postulate may, in part, account for recidivism after initial clinical improvement, which is occasionally observed following chemonucleolysis.7,41

The analogy between epidural corticosteroid and intradiscal chymopapain administration has obvious limitations. The sites of injection and the tissues targeted by the two therapeutic modalities are substantially different. Corticosteroid medications are deposited directly into the epidural space, preferentially in the region of the inflamed lumbar nerve root.41 Chymopapain is injected into the nucleus pulposus and is intended to exert its proteolytic action within this confined space. However, numerous investigators have documented that substances (including chymopapain) injected into injured discs frequently extrude through fractures in the posterior annulus and gain access to the epidural space, where they may contact spinal nerve roots at the injected level.7,36,52,59,62 Thus, although the intent of chymopapain chemonucleolysis is local treatment of the nucleus pulposus, in reality the enzyme’s therapeutic activity may extend well beyond the confines of the intervertebral disc space.

Several as yet unanswered questions are apparent. What proportion of active chymopapain injected intradiscally reaches the nerve root in the epidural space? How much is required to yield a clinically significant antiinflammatory effect (what is the dose–response curve)? Is the amount sufficient to incite tissue damage in the neural and/or soft-tissue elements (what is the therapeutic window)?

This latter query may be addressed, in part, by citing the results of previous investigations that have endeavored to assess the response of neural tissue to direct chymopapain administration.8,35,40,50,61 Zook and Krobine62 observed histological findings that suggested degenerative neuropathy when high-dose chymopapain (750 pKat U) was injected into the lumbar nerve roots of primates; no such changes occurred when the root was simply bathed in a similar concentration of the enzyme. Mackinnon and colleagues15 noted that an intrafascicular injection of chymopapain produced dose-related nerve fiber damage in both rats and primates. However, no nerve fiber damage occurred when similar concentrations were injected extrafascicularly (that is, into the epineurium) in either species. In the present study, topical application of chymopapain (100 pKat U) to the rat sciatic nerve created no histological evidence of axonal degeneration, demyelination, microvascular damage, or inflammatory reaction. In summation, there is no conclusive experimental evidence to suggest that the egress of chymopapain into the epidural space following intradiscal injection would induce adverse neurological sequelae. Clinical experience seems to corroborate this impression. In a review of over 400 complications in 13,000 patients treated with chymopapain, Watts48,59 identified no case in which damage to extradural nervous tissue was implied.

Role of PLA₂ as an Inflammatory Mediator in Discogenic Sciatica

This investigation provides evidence to support the hypothesis that chymopapain exhibits antiinflammatory properties in vivo. In an established model of acute inflammatory sciatica, a solution containing 100 pKat U chymopapain inhibited PLA₂ activity within an irritated nerve segment. Although our comprehension of the role of PLA₂ in initiating and maintaining the inflammatory response is still evolving, this enzyme has been implicated as a catalyst for inflammation in several human and animal models.5,14–17,34,45,47,49 Phospholipase A₂ belongs to a class of esterases that hydrolyze phosphoglyceride molecules within cell membranes, liberating a free fatty acid and a lysophospholipid.41 This represents the predominant pathway for arachidonic acid release, which in turn is the substrate for a cascade of enzymes that ultimately generate prostaglandins, leukotrienes, and thromboxanes.43 The liberation of arachidonic acid through PLA₂ activation is a key event in the synthesis of these potent proinflammatory lipid mediators.

In addition to its role in the arachidonic acid cascade, PLA₂ appears to act directly as an inflammangen, inciting inflammatory hyperemia, increased membrane permeability, histamine release, and edema formation both in vivo and in vitro.43,45,49 This group of enzymes also modulates the cell-mediated inflammatory response, binding and activating membrane receptors of neutrophils, T lymphocytes, mast cells, and tissue monocytes.43 Thus, a quantitative assay of PLA₂ activity in tissue facilitates an estimation of the magnitude of the inflammatory response and reflects both its cellular and humoral components.

Recent experimental evidence indicates that PLA₂ may play a prominent role in lumbar intervertebral disc disease. Saal, et al.49 identified extraordinarily high concentrations of PLA₂ in human prolapsed lumbar disc material and implicated this enzyme in the pathophysiology of discogenic radiculopathy. Subsequently, Ozaktay and associates45 documented that exogenous PLA₂ induced dose-dependent electrophysiological changes in rabbit lumbar dorsal nerve rootlets, resulting in the excitation, sensitization, and recruitment of previously silent nerve units. These data indicate that PLA₂ is capable of altering nerve responses in vivo. Additionally, PLA₂ created an acute inflammatory reaction in the facet joint capsules and paraspinal tissues, even when administered in relatively small concentrations.

The present study demonstrates that, in the modified Bennett and Xie model,2,38,39 experimental inflammatory neuropathy is coincident with a dramatic increase in PLA₂ activity. Variations in PLA₂ activity over time mirror the behavioral changes associated with acute sciatica, further substantiating the notion that PLA₂ activity is a reliable marker of inflammatory activity in vivo. Furthermore, enzyme activity is reduced by a known antiinflammatory agent (betamethasone) and by chymopapain.

Conclusions

This investigation has generated the following novel and/or remarkable findings: 1) direct topical administration of chymopapain to rat sciatic nerve segments does not
Chymopapain-induced reduction of phospholipase A₂ activity

produce histological evidence of neurotoxicity; 2) sciatic nerve ligation with loose chromic gut ligatures induces a marked increase in local PLA₂ activity over time in the rat; 3) variations in PLA₂ activity are coincident with the behavioral changes of acute sciatica in this model, including gait and postural abnormalities, guarding behavior, and mechanical hyperalgesia; 4) perineural infusions of chymopapain and betamethasone are equally effective in ameliorating these behavioral changes; and 5) perineural administration of chymopapain or betamethasone (but not saline) significantly reduces activity of the proinflammatory enzyme PLA₂. Further investigation is required to delineate fully the mechanism(s) of action responsible for the efficacy of chymopapain chemonucleolysis in vivo. However, the data derived from the present study indicate that chymopapain exhibits antiinflammatory properties that may be clinically relevant, particularly in the acute postnucleolysis period.

References
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