Collagenase in the Treatment of Dupuytren’s Disease:
an In Vitro Study

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The effects of clostridial collagenase on the tensile strength of Dupuytren’s cords was studied in vitro to assess its potential efficacy as an agent for clinical enzymatic fasciotomy. Collagenase was injected into Dupuytren’s cords from patients undergoing fasciectomy. Following a pilot experiment, in which a 3,600-unit dose of collagenase induced a 93% decrease in tensile modulus as compared with control cords, groups of five cords each were injected with 150, 300, and 600 units. These cords and a control group of five cords were tested by loading to failure in tension. The ultimate stress and strain to failure were recorded by a video capture technique. All specimens were stained for histologic examination with hematoxylin and eosin and for collagen typing with sirius red. Comparison of the ultimate stress values obtained with published values of extensor forces obtainable by the individual fingers of 40 normal hands indicated that a 300-unit dose of collagenase was sufficient for cord rupture within the average maximum force limits of the extensors of the index, long, ring, and small fingers (p < .02). All samples were in the residual disease stage histologically and contained type I collagen by sirius red staining. These results indicate that collagenase may be effective in enzymatic fasciotomy of residual-stage Dupuytren’s disease. (J Hand Surg 1996; 21A:490–495.)

Currently the most common therapy for Dupuytren’s disease is surgical fasciectomy. Several authors have proposed alternate modes of therapy, involving chemical or enzymatic fasciotomy. Investigators have studied the nonsurgical use of vitamin E, dimethyl sulfoxide, tocopherol, x-ray treatment, and physical therapy, with no resultant therapeutic benefit and very limited follow-up data.1–6 Case reports describing the use of methylhydrazine and allopurinol have shown some benefit,7,8 although long-term studies of these treatments have not been made. Steroids have been used both as a surgical adjunct9 and nonoperatively (Ketchum, LD, ASSH correspondence Newsletter No. 2, 1983). Steroids have shown promising results but have not been widely used as a nonsurgical treatment modality.

In 1971, Hueston10 reported the injection of a mixture of trypsin, hyaluronidase, and lidocaine, with cord rupture on subsequent forcible finger extension. This technique, which he termed enzymatic fasciotomy, was initially described in 1965 by Bassot.11 Full passive extension was obtained in all patients 15 minutes after injection. However, McCarthy,12 using this same technique, found that the initial preoperative deformity was again present in 75% of patients at 2- to 3-year follow-up examination. McCarthy12 concluded that there was

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Supported in part by Advance Biofactures Inc., Lynbrook, NY.

Received for publication March 20, 1995; accepted in revised form July 9, 1995.

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a similar rate of recurrence between surgical fasciectomy and enzymic fasciotomy but that because of the greater morbidity of enzymatic fasciotomy, its use offered no advantage over surgery.12

The present study was performed to assess three aspects of the potential clinical use of clostridial collagenase injection for Dupuytren's disease. First, we sought to define the collagenase dose range for clinical utility. Second, the defined dose range of injected collagenase was studied in vitro to determine the minimum effective dose capable of causing complete cord rupture. Finally, the force required to rupture cords was compared with the force physiologically attainable in extension of the average human hand. This information is crucial for determining the potential clinical efficacy of collagenase as a nonoperative therapeutic modality for Dupuytren's disease.

Materials and Methods

Tissue Preparation

For the first study phase using 3600-unit doses of collagenase, 20 Dupuytren's cords were obtained from patients undergoing fasciectomy. The specimens were randomly assigned to the collagenase injection group or the control buffer injection group, with 10 in each group. Seven men and three women supplied the cords in the collagenase injection group, and eight men and two women supplied the controls. The mean age in both groups was 61 years. The specimens were placed in saline-moistened gauze and immediately frozen at -70°C until testing.

In the second segment of the study, 20 cords were obtained from Dupuytren's patients at surgery. The specimens were randomly assigned to one of four groups: 150 units collagenase, 300 units, 600 units and a control buffer group. Each group contained five cords. All cords were supplied by men. The mean patient age for the 600-unit group was 62 years, for the 300-unit group 58 years, for the 150-unit group 67 years, and for the control group 58 years. The specimens were placed in saline-moistened gauze and immediately frozen at -70°C until testing.

Collagenase Injection

Prior to injection, the cross-sectional area of the cords was measured by a double-caliper technique at two locations in the injection area. These areas were then averaged for a final area value. Two india ink parallel marker lines were applied with the back side of a scalpel blade to the surface of the tissue to demarcate the injection treatment area.

Purified clostridial collagenase (Nucleolysin, Advance Biofactures, Lynbrook, NY) was buffered in a solution of 0.2 mM calcium chloride and 0.9% sodium chloride and appropriately diluted with this buffer. Ten cords were injected in the midsubstance with 3600 units of clostridial collagenase, of 0.5 mL total volume, by using a 25-gauge needle. Ten control cords were injected with 0.5 mL buffer only. The cords were then placed in a humidifying chamber and incubated at 37°C for 24 hours. Next, the cord ends were placed in titanium clips (Hemoclip, Edward Weck & Co., Research Triangle Park, NC).

For the multiple dose range study, it was necessary to anchor the cords by a method that would permit biomechanical testing to failure. Both ends of the cords were anchored to 0 polypropylene (Prolene) suture (Ethicon, Somerville, NJ) with a tapered CT-1 needle by the locking loop Krackow suture technique at both ends.13 This suture is nonabsorbable and not subject to degradation or weakening by the action of tissue enzymes. The mean tensile strength of the suture is 8.5 ± 0.5 kg (Ethicon, Inc., personal communication, August 1994). The cords were injected in the midsubstance, as described, with either buffer or 150, 300, or 600 units clostridial collagenase, of 0.2 mL total volume, and incubated as described for the pilot experiment.

Mechanical Testing

The cords were connected to a mechanical testing device (Chatillon, Greensboro, NC) via the titanium clips (3600-unit experiment) or the Prolene suture (multiple dose range study), and a load was applied. For the 10 cords injected with 3600 units of collagenase and 10 control cords, the samples were stretched to 10%, 15%, 20%, and 25% of their initial length but not to failure. Stress and strain recordings were made every 15 minutes until equilibrium was obtained at 1 hour for each of the stretch values. For the cords studied with a multiple collagenase dose range, we used a constant rate of 0.9 mm/s displacement of the cord ends. The resultant load was measured from a load cell placed in series with the cord tissue. The cord ends were displaced until cord rupture.

For all cords, tissue stress and strain were evaluated with reference to the two india ink marker lines on the specimen by a noncontact optical technique similar to that described by Woo et al.14 High-magnification images of the sample were continuously
videotaped during testing through a Zeiss 12.5× dissecting microscope fitted with a Telestill Photo Adapter (Designs for Vision, New York, NY). These images were then transferred to computer (Macintosh IIX, Apple Computer, Cupertino, CA) by using a video capture board (Rasterops, Santa Clara, CA) (Fig. 1). The distances between the two ink lines at six points along their length were evaluated at 1-second intervals until equilibrium was reached in the first experiment or until cord rupture in the second experiment with use of a software analysis package (Macmorph, Stony Brook, NY). This method allowed reliable measurement of stress and strain while eliminating the effects of slippage of the titanium clips or the suture at the cord ends.14

Histologic Analysis

All samples were sectioned at -20°C with use of a cryostat, and 6-µm sections were cut in longitudinal orientation, mounted on glass slides, and stained with hematoxylin and eosin for histologic typing and with 0.1% sirius red15 for collagen typing. The sections were examined and photographed under a Leitz-Dialux microscope (E. Leitz, Rockleigh, NJ). The hematoxylin/eosin samples were evaluated for disease stage (proliferative, involutional, or residual). Disease stage was also determined by the collagen type of the cords by viewing the sirius red-stained sections under polarized light. By this technique, type I collagen stains red, type II stains yellow, and type III stains green.16

Statistical Analysis

The tensile modulus was calculated by linear regression of the stress and strain data. At 10%, 15%, 20%, and 25% strain, samples in the 3600-unit collagenase study were compared with controls by Student’s t-test. Sample groups in the multiple dose range study were analyzed by regression analysis and the Wilcoxon rank sums test with a 95% confidence interval.

Results

In the first experiment, cords in the control buffer injection group had a mean tensile modulus of 33.017 ± 22.93 Mpa. In contrast, the mean tensile modulus of cords in the 3600-unit collagenase injection group had been decreased to 2.16 ± 3.20 Mpa (p < .002) (Fig. 2). This was a 93% decrease in tensile modulus after collagenase injection as compared with the controls. In addition, complete disruption occurred in 3 of the 10 specimens in the 3600-unit collagenase injection group but in none of the control specimens. The complete disruptions manifested differently during testing. In one case, upon removal of the specimen from the incubator, there was no longer continuity between the proximal and distal portions. In the other two cases, discontinuity occurred during the application of a specified strain and was immediately accompanied by a drop in the stress value to zero.

In the multiple dose range study of control, buffer-injected samples and samples injected with 150, 300,
and 600 units of collagenase, the stress to failure in all groups differed significantly. A curvilinear trend showed decreasing stress to failure with increasing collagenase dose (Fig. 3). After regression analysis, the $r^2$ value was 0.89, indicating that 89% of the trend was explainable by the differences in collagenase dose alone.

The stress to failure values were compared with the average extensor stress that each finger in the normal hand would be able to generate to simulate the clinical situation in which a patient is injected with collagenase and then asked to extend the finger(s) to rupture the cord. These values were obtained by averaging the cross-sectional area of cords and were compared with the average muscle tendon extensor force of each finger as reported by Ketchum et al.\textsuperscript{a7} Our analysis showed that 300 units of collagenase was the minimum effective dose sufficient to cause cord rupture within the normal extensor forces of the index, long, ring, and small fingers ($p < .02$) (Fig. 3).

Histologic analysis showed that all samples tested were in the residual disease stage according to criteria summarized by Shum.\textsuperscript{18} No nodules containing myofibroblasts were identified. Sirius red-stained sections revealed that all the cord tissue contained type I collagen. Type III collagen, which is typical in nodules, was not present in any sample. Control buffer-injected cords showed microtears in the collagen bundle structure (Fig. 4A). After collagenase injection, collagen lysis was apparent (Fig. 4B). The extent of collagen lysis was qualitatively increased with increasing collagenase dose.

**Discussion**

Our study demonstrates the ability of collagenase to decrease the tensile modulus and to rupture Dupuytren's cord tissue in vitro. To determine a minimum effective collagenase dose for potential clinical use, we first determined that a dose of 3600 units was far in excess of that needed for cord rupture in Dupuytren's disease. Next, in a multiple-dose in vitro study, we found that 300 units of injected collagenase, with a total volume of 0.2 mL, was the minimum effective dose able to cause cord rupture in the index, long, ring, and small fingers, based on the reported extensor forces of the individual fingers in the normal hand.\textsuperscript{17}

The clinical use of collagenase in the treatment of collagen disorders has been underway for many years. The collagenolytic properties of clostridial filtrates\textsuperscript{19} were first described in the 1940s. Clostridial collagenase has been shown to specifically degrade all collagen types in porcine dermal connective tissue models.\textsuperscript{15} In 1972, Mandl\textsuperscript{19} described the use of topical collagenase in the treatment of severe burns and in the treatment of leg ulcers. Its safe and effective use in the treatment of Peyronie's disease has been well demonstrated. In clinical trials by Gelbard et al.,\textsuperscript{20-22} a mean dose of 2695 (range, 1739–4850) units was injected into penile plaques. The safety of injected collagenase was a significant clinical finding of these trials. No patient showed clinical or laboratory evidence of systemic side effects, neural or vascular damage, or skin effects.\textsuperscript{20-22}

Hamilton et al.\textsuperscript{23} studied the immune responses of patients being treated with collagenase for Peyronie's disease. In this study, serum specimens from patients with Peyronie's disease were analyzed for collagenase-specific immunoglobulin E. Serum from only 1 of 44 patients contained detectable collagenase-specific immunoglobulin E following treatment. These authors concluded that an immune or anaphylactic reaction to injected collagenase would be unlikely.
Rydevik et al. showed experimentally that collagenase had no significant detrimental effect on nerve or vascular tissue in animal models.

Before collagenase may be considered for clinical use in Dupuytren’s disease, safety aspects must be well studied. The dose used in our study is well below the doses currently used clinically for Peyronie’s disease and is well below the doses studied for adverse nerve and vascular reactions in animal models.

It is also important to note that in all previous attempts to use chemical agents as nonsurgical treatments for Dupuytren’s disease, there was no distinction regarding the stage of the patient’s disease. The rationale for testing only residual-stage cord tissue in our study was to minimize early recurrence or worsening of the flexion contracture in patients such as might occur with collagenase injection of nodules if collagenase is not cytotoxic to nodular myofibroblasts, even though nodular collagen would be lysed. We speculate that this is the reason for the failure of prior studies with chemical agents. Future clinical study, with adequate long-term follow-up evaluation to assess local and systemic effects, is needed to determine the safety and efficacy of injected collagenase in Dupuytren’s disease. However, the use of collagenase for enzymatic fasciotomy in the treatment of residual-stage Dupuytren’s disease may be a suitable alternative to surgical fasciectomy in selected patients.

The authors thank Mr. Thomas Wegman and Dr. Judith Hirst of Advance Biofactures Inc. for generously supplying collagenase used in this study and for technical support regarding its use.

References