Effects of Streptokinase and Deoxyribonuclease on Viscosity of Human Surgical and Empyema Pus*

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Study objective: To investigate the effects of streptokinase and deoxyribonuclease (DNase) on the viscosity of pus to assess whether the DNase in the old preparation of streptokinase-streptodornase used intrapleurally to treat empyema was contributing to easier drainage of pus compared with purified streptokinase.

Design: In vitro measurement of pus viscosity.

Patients: Pus from three patients with surgically drained soft tissue abscesses and from six patients with empyema thoracis of varying etiology was studied.

Interventions: Pus samples were incubated with saline solution as control and with streptokinase, streptokinase-streptodornase, human recombinant DNase, and a mixture of streptokinase and DNase in concentrations approximating those achieved in clinical practice.

Results: Purified streptokinase had little effect on pus viscosity, with a mean reduction of 11.1% in the surgical specimens and 1.7% in the empyema samples. Streptokinase-streptodornase reduced viscosity by a mean of 52.8% in the surgical samples and 94.8% in the empyema samples. Human recombinant DNase reduced viscosity by a mean of 32.7% in surgical samples and 93.4% in empyema samples. Adding streptokinase to human recombinant DNase produced no further reduction in viscosity. Final viscosities in samples treated with DNase were very similar whatever the starting viscosity.

Conclusions: DNase significantly reduces pus viscosity, whereas streptokinase has little or no effect, and in empyema may work simply by breaking down loculations. Clinical studies should be undertaken to see if these in vitro changes produce clinical benefits. The simple viscometer devised for these experiments may also prove useful in other contexts.

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Key words: deoxyribonuclease; empyema thoracis; pus; streptokinase; viscosity

Abbreviation: DNase = deoxyribonuclease

Infection of the pleural space with the formation of pus in the pleural cavity remains a serious and life-threatening condition despite modern antibiotics. Historically, the condition was recognized by Aristotle1 and Hippocrates,2 and the importance of adequate drainage of the pleural space was recognized in ancient times. Closed drainage of the chest via tube thoracostomy was originally described in 1875, but came into more widespread use after being reported by Graham in 1918.2

It has long been recognized that infected fluid in the pleural space may be difficult to drain without surgical intervention, because as the fluid becomes more frankly purulent, it tends to loculate by formation of fibrinous strands and to become more viscous and adherent to the pleural surfaces. It is over half a century since Tillett and Sherry3 attempted to overcome these problems and increase the effectiveness of tube drainage by intrapleural instillation of enzymes derived from streptococci. They extracted enzymes from concentrated filtrates of hemolytic streptococci of Lancefield group C. The strain used was potent in its production of both streptokinase and deoxyribonuclease (DNase), and the preparations used in the classic article3 contained both enzymes in varying quantities. Because of the impure nature of their extracts, some allergic reactions
were observed, but they did demonstrate improved drainage and also that the viscosity of pus in the pleural cavity was reduced after intrapleural instillation of their preparation.

In later years, a commercial preparation was marketed consisting of streptokinase 125,000 U/mL and streptodornase (streptococcal DNase) 12,500 U/mL. This preparation has been extensively used in the topical treatment of necrotic ulcers. In the United Kingdom, the preparation was also used for intrapleural treatment of empyemas, and one of us (G.S.) had extensive experience with this. Outside the United Kingdom, because of concern about allergic reactions, the combined preparation fell into disuse, but with the increased availability of more highly purified streptokinase produced for cardiological use, there was a resurgence of interest in intrapleural enzyme therapy in the 1980s and 1990s. There are several uncontrolled series supporting the use of fibrinolytics.3–7 Two controlled randomized studies of intrapleural thrombolitics gave conflicting results.8,9 The consensus is that intrapleural streptokinase alone is of some benefit in treating empyema, and it is widely used. Reviews have suggested that fibrinolytics may reduce pus viscosity and increase drainage.10 Indications for use include not only loculation, but also “the presence of thick or viscous pus.”11 However, it has been the firm clinical impression of one of us (G.S.) that the newer purified preparations are much less effective than the older preparation. As it has been known for many years that empyema pus has a very high content of DNA,12 we postulated that the DNase in the older preparation was contributing to its effectiveness by reducing the pus viscosity, and thus, improving drainage.

Human recombinant DNase is now available, and this could offer another therapeutic avenue in the treatment of empyemas without the danger of the allergic reactions that caused problems with the crude streptococcal extracts. In the development of human recombinant DNase for use in reducing viscosity of sputum in patients with cystic fibrosis, Shak et al13 originally described the “pourability” assay. Sputum was simply poured from one small plastic tube into another before and after incubation with DNase. We initially attempted to measure the “drainability” of pus and the effects of incubation with various enzymes on drainability with the simple device described below.14 Further analysis, however, showed that this device could actually be used to measure viscosity. We therefore used the device to assess the effects of incubation with streptokinase, streptokinase-streptodornase, and human recombinant DNase on viscosity of empyema pus.

Materials and Methods

Measurement of Viscosity

The device for measuring drainability was simply a 3-mL medical syringe (Terumo Medical, Elkton, MD) fitted with an 18-gauge, 1.2 × 38-mm nonbevelled drawing-up needle (Becton Dickinson Medical; Singapore; Fig 1). Initial studies consisted of filling the syringe to the 3-mL mark and counting the drip rate of the fluid from the needle.14 However, absolute measurements of viscosity can be made from this simple device.

Theoretical Basis

The resistance to flow in a pipe due to viscosity depends on the radius to the fourth power. The barrel of the syringe has a radius that is about 10 times that of the needle, so it is a good approximation to ignore the effect of the barrel when we are considering viscous flow through the syringe. By considering the shear force per unit area on a cylindrical surface of fluid inside the needle lumen, it can be shown that the flow (dv/dt) through the needle is:

\[
\frac{dv}{dt} = \frac{\pi r^4 \Delta P}{8 L \mu}
\]

where \(r\) is the radius of the needle, \(L\) is its length, \(\Delta P\) is the pressure difference between the two ends, and \(\mu\) is the viscosity.

In our application, the supply of fluid to the needle is from the reservoir in the syringe barrel above the needle, and the pressure difference across the needle is \(rh\) where \(\rho\) is the density of the fluid, \(g\) is the gravitational acceleration, and \(h\) is the height of the fluid in the syringe above the top of the needle as illustrated in Figure 2. Because the level in the syringe changes, so does the

Figure 1. Simple bench-top viscosometer.
pressure applied to the slow flow in the needle. Introducing \( R \), the radius of the barrel of the syringe, we can link the volume change in the syringe to the volume of flow through the needle and obtain:

\[
h_2 = h_1 \exp\left(\frac{r^4 \rho g t}{8L \mu R^2}\right)
\]

Where \( h_1 \) is the starting height of the fluid above the top of the needle, and \( h_2 \) is the height after \( t \) seconds. The viscosity is then obtained directly from:

\[
\mu = \frac{r^4 \rho g t}{8L R^2 \ln(h_2/h_1)}
\]

To calculate viscosity by counting the drip rate from the syringe, the starting height \( h_1 \) is recorded and the number of drops falling during the interval of \( t \) seconds is counted. It is then necessary to calibrate the drop size (by measuring the volume of a known number of drops). If the drop size is \( v \), then:

\[
h_2 = h_1 - \frac{vN}{\pi R^2}
\]

This can be incorporated into the previous equation and viscosity obtained.

**Patients**

Three samples of surgical pus were studied and six from patients with empyema thoracis (see Table 1).

**Incubation of Samples**

Each 4-mL aliquot of pus was incubated for 4 h with added streptokinase, streptokinase-streptodornase, human recombinant DNase, and with a mixture of streptokinase and human DNase. Enzymes were diluted so a total volume of 0.4 mL was added to each 4-mL aliquot of pus, and a control sample was incubated with 0.4 mL of normal saline solution. Enzyme concentrations of streptokinase and of streptokinase-streptodornase were calculated to reflect the concentration of enzyme that would be obtained \textit{in vivo} by injecting standard doses of streptokinase (250,000 U) into a 1-L empyema. For a 4-mL aliquot that equals 1,000 U of streptokinase, the streptokinase-streptodornase preparation thus contained 1,000 U of streptokinase with 250 U of streptodornase.

Dornase alfa contains 1,000 U/mg of DNase activity, and doses from 20 to 100 \( \mu \)g were added to the aliquots of pus. Adding 20 \( \mu \)g of dornase alfa to 4 mL of pus gives a concentration of enzyme equivalent to injecting two 2.5-mg ampoules of dornase alfa into a 1-L empyema. The initial experience with surgical pus and with the first empyema sample (Fig 3) suggested there was little dose-response relationship using dornase alfa, and in subsequent experiments, 40 \( \mu \)g dornase alfa was used as standard.

**Results**

Table 2 and Figure 4 give the results on the surgical pus specimens. Streptokinase produced minor reductions in viscosity, whereas streptokinase-streptodornase reduced the viscosity of all specimens by approximately 50%. Different concentrations of dornase alfa were used in the three cases (100 \( \mu \)g in case 1, 20 \( \mu \)g in case 2, and 40 \( \mu \)g in case 3). There did seem to be some suggestion that the higher

<table>
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<tr>
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<td>Ischiorectal abscess</td>
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<td>Patient 3</td>
<td>Soft tissue abscess</td>
</tr>
<tr>
<td>Empyema pus</td>
<td></td>
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<tr>
<td>Patient 1</td>
<td>Staphylococcal empyema after rupture of lung abscess</td>
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<td>Patient 2</td>
<td>Tuberculous empyema with bronchopleural fistula</td>
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<td>Empyema secondary to malignant bronchopleural fistula after pneumonectomy</td>
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<td>Patient 5</td>
<td>Empyema following pneumococcal pneumonia</td>
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<td>Patient 6</td>
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concentrations of dornase alfa were slightly more effective, however. Figure 3 shows the dose response using dornase alfa in the first empyema specimen, which suggested little or no increase in efficiency with increasing dose.

Results of the empyema specimens are shown in Table 3 and graphically in Figure 5. Streptokinase had no significant effect on pus viscosity in any sample. Streptokinase-streptodornase and dornase alfa both reduced viscosity dramatically to around 0.005 Pa seconds whatever the initial viscosity. The combination of dornase alfa and streptokinase was no better than dornase alfa alone, which produced results very similar to streptokinase-streptodornase.

**Discussion**

We have demonstrated that purified streptokinase has minimal effect on reducing the viscosity of pus in humans. The effect is perhaps marginally greater in the samples of surgical pus than in the empyema pus. This could be because of greater contamination of the surgical samples with blood. There are few data on effects of streptokinase on pus viscosity, although some authors imply that this is part of the way by which it exerts its effect. Park et al assayed the effect of urokinase on the viscosity of a variety of body fluids including abscess fluid and found that viscosity was decreased by 23% by urokinase. The combination of streptokinase and streptococcal DNase produces marked reductions in viscosity, and these can only be attributed to the presence of the streptodornase. Results with human recombinant DNase are similar, and the absolute viscosities achieved are remarkably similar in all samples whatever the starting viscosity. Dornase alfa thus proportionately decreases viscosity much more dramatically in samples of initial high viscosity. The addition of streptokinase to DNase produces no further additional reduction in viscosity in the empyema samples. There was little difference between the effects of streptokinase/streptodornase and human recombinant DNase in terms of final viscosities achieved, supporting the suggestion that it is DNA content that determines viscosity.

The specific activity of dornase alfa in human recombinant DNase is 1,000 U/mg (A. Corder, BSc; personal communication; July 10, 1997), whereas that of streptodornase is 2,400 U/mg. However, it is not clear that the two preparations have been assayed under identical conditions. In practical terms,

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*See Figures 3, 4 for abbreviations.
However, human recombinant DNase would seem to be just as effective as streptodornase in reducing pus viscosity in the more viscous samples.

When one considers an infected pleural cavity, there is no doubt that one major obstacle to effective drainage is the formation of fibrinous locules, and there can be little doubt that fibrinolytic enzymes will prove to be the treatment of choice for this complication. However, Tillett and Sherry in 1949 recognized that there were a number of ways in which pleural infections could develop, and spoke of “fibrinous, purulent and sanguinous pleural exudations.” It is not difficult to imagine that once pleural fluid has turned into frank pus, which is viscid and sticky, that simply removing loculations will not necessarily lead to efficient drainage. We may for half a century have been ignoring the important contribution of the DNase component of the extracts used by Tillett and Sherry in improving empyema drainage. The combination of streptokinase and streptodornase has been shown to be more effective than proteolytic enzymes in cleaning and desloughing infected superficial ulcers and this combination may also be effective in cleaning out debris from an infected pleural cavity.

All the specimens used in this study were frankly purulent (stage 6 or 7 in Light’s classification), and we do not suggest that similar results would be obtained in the earlier stages of the development of parapneumonic effusions into empyemas. However, if fluid is purulent, there may be a place for use of DNase in treatment, although this should take place in the context of a proper controlled clinical study.

DNase could also have a place in percutaneous drainage of abscesses in other sites in the body, as this is increasingly being done with the advent of the interventional radiologist. One could even speculate that enzyme therapy could be tailored on the basis of measurements of viscosity that could be done at the bedside in a matter of minutes by use of the device described here. The calculation looks difficult, but is easily done using a computer spreadsheet. Alternatively, a threshold value for use of DNase may become evident, in which case this can simply be translated to a simple count of drip rate from the syringe. Measurement of pleural fluid viscosity could also be another useful parameter in the classification of the development of parapneumonic effusions.

We suggest that these preliminary findings warrant inclusion of an arm incorporating DNase as intrapleural therapy in future trials of enzyme treatment of empyema.

**REFERENCES**


<table>
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<tr>
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*See Figures 3, 4 for abbreviations.*
19 Light RW. Pleural diseases. 3rd ed. Baltimore, MD: Williams & Wilkins, 1995; 142