Occasional reviews

The role of recombinant human DNase in the treatment of patients with cystic fibrosis: many promises, more problems

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The median survival of patients with cystic fibrosis at present approaches 30 years.¹ Essentially, the ongoing medical and scientific struggle towards further improving this prognosis relies on the continued search for new treatment strategies and novel therapeutic substances. A relatively recent development is recombinant human DNase (rhDNase). The clinical efficacy of rhDNase has been discussed at scientific and educational meetings for several years, and it has been introduced into the therapeutic routine of many cystic fibrosis centres via large multicentre clinical trials. Thus, most cystic fibrosis caregivers have had ample opportunity to familiarise themselves with rhDNase, its safety profile, and its therapeutic effects.

Encouraging short term improvements in lung function, hopes that focus on any new and promising development, and considerable interest and pressure from cystic fibrosis lay organisations have motivated many centres to support national registration and to prescribe rhDNase to their cystic fibrosis patient populations. On closer scrutiny, however, there remains a spectrum of as yet unanswered questions regarding the long term effectiveness of rhDNase, indications for a more targeted prescription, and possible interactions with other treatment strategies. Recently published review articles either emphasise the problems or the promises of this new treatment strategy and thus differ substantially in their messages.^{2,3} This review, written from a clinical perspective, analyses the available data to achieve a more balanced view on the present role of rhDNase in the management of patients with cystic fibrosis.

Background

The DNase story is, in fact, not a new one. In the 1950s a series of studies showed that the viscoelastic properties of respiratory secretions in cystic fibrosis largely depend on their DNA content.⁴⁻¹⁰ It was shown that high levels of lung infection in cystic fibrosis are associated with markedly raised sputum DNA levels which mainly stems from the patient's own neutrophils.^{8,10,11} Some of the earlier work had already shown that the rheological properties of sputum from patients with cystic fibrosis

could be altered by adding DNase.^{4,12,13} This again indicated the possibility of a new therapeutic strategy for improving the clearance of mucus from the airways of patients with cystic fibrosis.

Clinical studies with bovine DNase commenced as early as 1953.¹⁴⁻¹⁷ These trials, however, were subsequently stopped because of significant adverse effects.¹⁸ Human DNase, an enzyme normally produced in the pancreas and salivary glands, was first successfully cloned, sequenced, and expressed in 1990.¹⁹ Clinical studies with this recombinant human DNase started in 1992 and results from the first phase 1 trials, conducted to determine the tolerance to rhDNase and to gather basic pharmacokinetic data, were published in 1992.^{20,21}

Meanwhile, the safety profile of the substance has been well established. Inhalation of rhDNase seems to have no serious side effects; an increased incidence of voice alterations, laryngitis, pharyngitis, dyspepsia and facial oedema has been observed in patients treated with rhDNase.^{3,22-24} Antibody formation against rhDNase is rare but can occur.^{23,25} Treatment with rhDNase appears to be safe enough for more severely diseased patients.²⁶

As with any other inhaled medication in cystic fibrosis, the therapeutic effect of rhDNase depends on using a suitable nebuliser. Considerable differences exist in aerosol qualities and respirable fraction between some of the most frequently used devices.²⁷ With effective inhalation technique and an appropriate nebuliser, the substance reaches the bronchial secretions and, as intended, reduces their viscoelastic properties by cleaving DNA.^{28,29}

This, however, does not necessarily mean that the biochemical effects of rhDNase are of clinical relevance. There are some theoretical caveats against extrapolating reduced viscoelastic properties of secretions into an obligatory increase in their clearance. Bronchial secretions are characterised by an optimal range of viscosity for effective mucociliary transport³⁰ and a further reduction in viscosity could lead to a progressive mechanical uncoupling of cilia and secretions. The transport of mucus by coughing depends not only on its viscoelasticity but also on its adhesive surface properties.³¹ A decrease in the surface tension of mucus is

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generally associated with a decrease in its adhesive properties and a resulting increase in the transport efficacy of coughing.³² It was recently shown that DNA fragmentation by rhDNase may decrease the surface tension of mucus and thus alter its adhesive properties favourably.³³ It follows that rhDNase might support cough clearance by its effects on the surface properties of mucus; at the same time, depending on the pretreatment viscosity of bronchial secretions, the substance could either enhance or reduce mucociliary clearance by its effects on viscoelasticity. While such theoretical considerations argue against a general therapeutic benefit by a reduction in viscoelasticity in all patients, further questions need to be asked from a more clinical point of view.

Short term effects

Here the question is whether rhDNase really enhances the clearance of abundant secretions. As with studies of chest physiotherapy, this effect might be demonstrated by quantifying expectorated sputum, observing the clearance of radioaerosols, recording lung function changes, and assessing the rate of relevant respiratory complications such as atelectasis. Early clinical studies to evaluate the short term effects of rhDNase all focused on lung function changes and reported significant and clinically relevant improvements in forced expiratory flow rates and volumes after a few days of treatment.^{21 22 34} Intermittent treatment with rhDNase resulted in promptly improved lung function which returned to baseline levels when inhalations were discontinued.³

To investigate the theory of a cause and effect relationship between increased sputum clearance and improved lung function a correlation between the weight of expectorated sputum and the dimension of observed lung function changes must be shown and, in fact, such a correlation has been reported in in-patients with cystic fibrosis in a previous chest physiotherapy study.³⁵ For rhDNase, however, studies of sputum weight and correlations between sputum weight and lung function have not, as yet, been performed. The extent of the changes in sputum viscoelasticity following treatment with rhDNase has been shown to correlate with changes in lung function.²⁹ One radioaerosol study indicated a marginally improved clearance after rhDNase.³⁶

In contrast to the lack of controlled data, many uncontrolled clinical observations have indicated that rhDNase does facilitate sputum clearance and many patients have reported that they find it much easier and more effective to raise secretions with the aid of rhDNase than without. Thus, presently established scientific evidence, together with everyday clinical experience, suggest that the observed changes in lung function are effected by enhanced clearance of secretions. It should be relatively easy to substantiate this hypothesis further in future clinical trials.

Long term effects

Here the question is whether the beneficial short term effects of rhDNase will translate

into therapeutic long term effectiveness. The rationale behind expecting a beneficial effect on the course of the disease from improved clearance of secretions stems from our present understanding of the mechanisms of the disease. The proteolytic activity in the bronchial secretions of patients with cystic fibrosis is significantly increased, and this excessive proteolytic activity mainly stems from the host's own neutrophils.³⁷ In addition to elastase, collagenase and peroxidase have recently been found in the bronchial secretions of bacterially infected patients with cystic fibrosis.^{38 39} This proteolytic load on the airway wall affects ongoing tissue damage and, thus, progression of bronchiectasis.³⁷ Consequently, one may expect that effective clearance of secretions should reduce this proteolytic burden and, thereby, favourably alter the course of the disease.

This hypothesis might be tested by observing long term changes in lung function, by counting acute exacerbations and hospital admissions, and by quantifying other necessary therapeutic interventions. Furthermore, any therapeutic long term effect on disease progression should improve quality of life and survival. So far, studies on the long term effects of rhDNase therapy have mainly focused on changes in lung function. These controlled and uncontrolled (observational) studies have ranged in duration from several months to two years^{23 25 26 40}; mean improvements of more than 10% in forced expiratory volume in one second (FEV₁) have been reported for the first days of treatment but a longer duration of treatment resulted in markedly smaller positive changes. Given this reduction in the effects on lung function with time, the question arises as to whether the therapeutic effectiveness might be insignificant with extended treatment. A recently published two year study which reported an improved outcome did not contradict this speculation as it was merely observational and lacked a control group⁴⁰; furthermore, several of the most severely diseased patients in this study succumbed and thus had no statistical input into the group outcome. Another recent paediatric six month study reported large interindividual differences in the effects of rhDNase on lung function, with some patients improving while others were unchanged or even deteriorated.⁴¹

Such large interindividual differences in the long term effects of rhDNase have been observed by many cystic fibrosis caregivers. Various attempts at predicting the outcome for the individual patient on the basis of pretreatment clinical data have failed⁴²; even assessment of the patient's daily sputum production before treatment with rhDNase did not correlate with subsequently observed effects on lung function.⁴³ While some patients report considerable improvement in their sputum clearance and others do not, this subjective assessment also fails to correlate with objective outcome measures.⁴¹

The effects of rhDNase on lung function not only decrease with time, but may be completely lost after the medication is terminated. One six month treatment study showed that, after

stopping rhDNase, lung function dropped markedly below the initial baseline level.²⁵ When this pretreatment baseline value is plotted against that end point a slope of deterioration is obtained that does not differ substantially from the natural course of the disease. Concern arises as to whether treatment with rhDNase only effected a superficial removal of secretions while, deeper down on the mucosal surface, tissue damage continued as before. If so, therapy would only have effected some "lung function cosmetics" while having no impact on the long term progression of the disease. In a recently reported study, rhDNase failed to reduce the level of elastase and other inflammatory markers in the sputum and blood of patients with cystic fibrosis.⁴⁴

Thus, a beneficial long term effect of rhDNase is not yet firmly established from the available literature on its effects on lung function. Less attention has been paid to other outcome measures, but one six month multi-centre study found a dose-dependent tendency towards fewer acute respiratory exacerbations with rhDNase treatment.²³

Supportive effects

Effective clearance of secretions should make the bronchial mucosa of patients with cystic fibrosis more accessible for other inhaled medications such as antibiotics, amiloride, topical steroids, proteinase inhibitors, and gene therapy. As with chest physiotherapy, rhDNase could thus have a potential role as a facilitator of other aerosol medications. Valid studies to support this hypothesis, however, are lacking. One radioaerosol study demonstrated a more homogeneous deposition of tracer after rhDNase.³⁶ Recent in vitro data indicate that rhDNase might be superior to other mucolytic agents in assisting the access of liposomal gene vectors to the cell surface,⁴⁵ but here the question arises as to whether or not the enzyme will damage the therapeutic DNA-liposome complex.

Another potential supportive role of rhDNase which needs to be investigated is the possibility that it improves the antibacterial efficacy of antimicrobial agents. Antibiotic binding to sputum has been described by several investigators, and sputum DNA plays an important role in this phenomenon.⁴⁶⁻⁵⁰ The few in vitro data available suggest that this binding to various sputum fractions can be reduced by adding DNase,⁵¹ which indicates the possibility of an interesting therapeutic synergism. Concern has arisen as to whether the combination of rhDNase and inhaled antibiotics could have other more adverse effects, but recently obtained results suggest that, even if this combination of inhaled substances did occasionally reach the alveolar space, the surface tension properties of pulmonary surfactant would not be significantly affected.⁵² In vivo findings, however, have so far remained inconclusive. A reduction in the colony density of several relevant bacterial invaders was reported in one observational study of patients with cystic fibrosis treated with rhDNase.⁵³

Other investigators, however, did not find a significant reduction in the serum level of anti-*Pseudomonas* precipitins after one year of treatment with rhDNase.⁵⁴ It follows that the possibility of rhDNase enhancing the efficacy of inhaled or systemic antibacterial medication is as yet insufficiently explored. Clearly, this potential effect of rhDNase is an interesting and important issue that calls for further study.

Prescription

If it is correct that rhDNase has some therapeutic long term effectiveness, when, in the course of the disease, should such therapy commence? Patients with full blown suppurative lung disease will certainly provide more than sufficient substrate for the enzyme. An unresolved question, however, relates to the administration of rhDNase in mildly or moderately diseased patients and those with early stages of the disorder. There are arguments both for and against early prescription.

In general, clinical and radiological assessment, as well as conventional lung function testing and sputum bacteriology, tend to underestimate the amount of bronchial inflammation and infection in patients with seemingly mild or moderate lung disease. This is illustrated by bronchoalveolar lavage studies where evidence for a neutrophil-dominated increase in cells and abundant active elastase was found in clinically stable patients with only mildly compromised lung function.⁵⁵ Others observed significantly increased DNA levels in the lavage fluid of infants with cystic fibrosis.⁵⁶ Based on similar lavage findings, some authors have even postulated that chronic inflammation of the bronchial mucosa may commence before bacterial infection occurs.⁵⁷ While this issue remains controversial, it is clear from the recent literature that mucosal inflammation may occur surprisingly early, and these findings would argue for a liberal and early prescription policy for rhDNase in clinical practice.

Such a liberal and early prescription policy, however, will, at least in some patients, result in unnecessarily high treatment costs. In addition, it could induce antibody formation in a few patients before their disease has progressed to a stage where they would really benefit from treatment. Furthermore, the question arises as to whether rhDNase could have any untoward effects in more mildly diseased patients who have so far maintained effective mucus transport. In an in vitro study using a frog palate model, rhDNase increased the transport velocity of those cystic fibrosis sputum samples with a decreased baseline mucociliary transport rate; however, it tended to decrease mucus transport velocity when added to other sputum samples with an increased baseline transport rate.³³ This finding indicates the possibility that rhDNase may occasionally have a negative effect on mucus transport in patients with only mildly damaged mucosa. The pathophysiological explanation for this negative effect could be the aforementioned mechanical uncoupling of ciliary action and the mucus layer.

Thus, there are arguments for and against the early prescription of rhDNase. Obviously, the routine management of patients with cystic fibrosis does not provide sufficient information for a targeted and individualised indication. The question arises as to whether data from more invasive investigations such as fibreoptic bronchoscopy and bronchial lavage should be considered as a prerequisite for prescribing rhDNase to mildly and moderately diseased patients.

Interaction with other therapeutic strategies

Other strategies for clearing excess bronchial secretions have a traditional position in the management of patients with cystic fibrosis, especially the various techniques of chest physiotherapy which have been developed to match the therapeutic needs of these patients specifically.⁵⁸ Although applying a mechanical rather than a pharmacological concept of treatment, chest physiotherapy pursues the same goal as rhDNase. Surprisingly, however, no clinical studies to compare the relative benefit from these two different approaches have been undertaken. One recent *in vitro* study demonstrated an optimal effect on sputum spinability when rhDNase and airflow oscillations were applied concomitantly.⁵⁹ In general, cystic fibrosis caregivers tend to consider chest physiotherapy and rhDNase inhalation as complementary rather than competitive, but this concept is based exclusively on uncontrolled clinical observations. However, some other clinical observations might suggest a different perspective on this issue. In some European cystic fibrosis centres doctors were surprised that chest physiotherapists were able to predict whether or not a patient would respond with an improvement in lung function to newly prescribed rhDNase. Such foresight was (and is) usually based on the physiotherapist's in-depth knowledge of the particular patient's chest physiotherapy skills and compliance. Thus, one is frequently able to predict a marked therapeutic response in those patients who produce significant amounts of sputum but comply badly with their daily chest physiotherapy routine. One can speculate that those recent studies that attempted to predict the lung function response to rhDNase of patients with cystic fibrosis^{42,43} had to fail because they did not take into account the expertise and compliance with chest physiotherapy of the subjects.

The possibility therefore remains that regular inhalations of rhDNase might effectively replace the daily chest physiotherapy routine in some patients; in others, chest physiotherapy may be more effective than rhDNase, while a third subgroup might actually benefit from the presently prevailing strategy of combining these two therapeutic approaches. Finally, one can speculate that even if maximum clearance of secretions could be achieved by chest physiotherapy alone, rhDNase would be a valuable adjunct if it made mechanical clearance less time and energy consuming. At present, however, all these speculations lack any scientific

background. It follows that the relative role of physical and pharmacological clearance of the airways in patients with cystic fibrosis needs to be more clearly defined by carefully conducted clinical studies.

The role of other mucolytic agents in the management of patients with cystic fibrosis has not been clearly established. There is the theoretical possibility of combining rhDNase with other mucolytic substances in an attempt to maximise their effects on viscoelasticity. Recent *in vitro* results indicate that combinations with nalcystelyn, gelsolin, and hypertonic saline are, in fact, more effective on the viscoelastic properties of cystic fibrosis sputum than rhDNase alone.⁶⁰⁻⁶² Translating these results into a clinical experiment might, however, carry the risk of ineffective ciliary beating in the presence of large intrabronchial pools of liquefied secretions; in the presence of an additionally resulting mechanical handicap for coughing and chest physiotherapy, secretions might be shifted from one lung region to another rather than expectorated, and negative effects on the mechanics of breathing and on gas exchange might ensue.

Conclusions

In conclusion, the present widespread clinical use of rhDNase in the treatment of patients with cystic fibrosis is burdened by a range of unresolved problems and open questions. It seems the substance might have been introduced into the clinical routine too early, and this enthusiastic haste is being paid for with doubts on the ethics and cost effectiveness.

A liberal policy of prescribing rhDNase to all but the most mildly diseased patients will burden the health care system with considerable costs, will further complicate the daily therapeutic routine of patients by one more aerosol administration and, with a small but nevertheless finite risk of side effects, will be a load on the conscience of the caregiver. On the other hand, a very restrictive general prescription policy will burden the caregiver with the risk of withholding potentially valuable therapy and, in addition, such a position will be complicated by pressure towards more liberal prescription from patients, parents, and lay organisations.

A more targeted approach, based on a careful assessment of the disease in individual patients, might appear to be a more reasonable alternative; however, this remains only a theoretical option as there are no established criteria for effectively individualising treatment at present. As already mentioned, attempts at predicting the lung function response of the individual patient on the basis of sputum production and various other clinical parameters have failed.^{42,43} Some groups promote the use of scoring systems, but these are based on arbitrarily chosen criteria and, so far, have not been validated.⁶³ Another frequently used method to individualise the prescription of rhDNase is a short therapeutic trial with closely monitored results. As outlined above, however, an easily assessed improvement in short term

lung function cannot be extrapolated into clinically relevant long term benefits. To rely on the patient's own subjective assessment of therapeutic efficacy is also questionable as some patients report beneficial effects even in the presence of objectively documented deterioration.⁴¹ In summary, it remains unclear whether or not treatment with inhaled rhDNase has a positive impact on the long term course of the disease; if it does, no clearly defined criteria are available to identify those who will benefit.

It seems that in many cystic fibrosis centres the initial enthusiastic welcome for a promising new substance is turning into the more sober acknowledgment of a therapeutic problem. Clearly, the present insight into the potential and limitations of inhaled rhDNase is far from complete and more studies are urgently needed.

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