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Translational Research in Pleural Infection and Beyond

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ABSTRACT

The incidence of pleural infection has been rising in recent years. Intrapleural therapy with tissue plasminogen activator (tPA) and deoxyribonuclease (DNase) has significantly reduced the need of surgery and its impact on clinical care is rising worldwide. Efforts are underway to optimize the delivery regime and establish the short and longer term effects of this therapy. The complex interactions of bacterial infection within the pleura with inflammatory responses and clinical interventions (antibiotics and tPA/DNase) require further studies to improve future treatment options. Intrapleural instillation of tPA potently induces pleural fluid formation, principally via a monocyte chemotactic protein (MCP)-1 dependent mechanism. Activation of transcriptional programs in pleural resident cells and infiltrating cells during pleural infection and malignancy results in the local secretion of a cocktail of pro-inflammatory signalling molecules (including MCP-1) within the pleural confines that contributes to effusion formation. Understanding the biology of these molecules and their interaction may provide novel targets for pleural fluid control.

OVERVIEW

Pleural infection is a centuries-old illness that has claimed many lives throughout the history of mankind. Hippocrates (460-375 BC) described seminal findings in empyema that stand firm as the principles of modern day care.[1] More than two millennia since Hippocrates' era, pleural infection continues to cause major morbidity and mortality. Many famous lives, and their contribution to the world, were cut short as they succumbed to pleural infection. The most documented case was Sir William Osler (1849-1919)[2], father of modern medicine, who died after a protracted course of empyema; he joined Guillaume Dupuytren, Karl Marx, Benjamin Franklin, amongst many others, as victims of this deadly disease.

Bender *et al* interrogated a century (1900-2005) of data from the state of Utah and showed that the highest mortality rate from empyema occurred in the early 20th century during the Spanish influenza epidemic and decreased dramatically since the introduction of antibiotics.[3] The mortality however has resurged significantly since the 21st century began. Many papers have confirmed similar rises in incidence and/or mortality of pleural infection around the world.[4] National data from the USA showed an alarming 200% (95% CI 180-210%) increase in hospitalized empyema rates between 1996 and 2008 (total hospitalizations =157,094)[5] The increases were observed among children (by 1.9 fold) and adults of all age groups: including those aged 18-39, 40-64 and over 65 years (by 1.8, 2.0 and 1.7 fold respectively) [5]. Although ~80% of pleural infection can be treated with antibiotics and tube drainage[6,7], these worrying data highlight the need of research and advances in treatment and, in the longer term, prevention of pleural infection.

The last decade has seen a significant rise in research in pleural infection with exciting breakthroughs. The discovery of combination intrapleural therapy using tissue plasminogen activator (tPA) and deoxyribonuclease (DNase) has revolutionized practice, curing the majority of patients without resorting to surgery.[8] The incredible story of the discovery of tPA/DNase started from a publication by Simpson *et al* in CHEST[9], followed by a remarkable journey of translational work through international collaborations integrating bench research and multi-center clinical trials. Our review highlights this heartening tale, and discusses ongoing and future research directions related to intrapleural therapy. Importantly investigations of the mechanisms of tPA/DNase have already opened up opportunities with far-reaching implications for other pleural diseases and beyond.

INTRAPLEURAL tPA/DNase THERAPY

'If an empyema does not rupture, death will occur' Hippocrates.

Medications for sepsis control and drainage of the infected pleural material were advocated in the *Hippocratic Corpus* - these foundations have stood the test of time as the cornerstones for treatment of pleural infection.

Hippocrates first described thoracostomy for patients who failed conservative treatment. He applied mud on the rib cage and performed open drainage at the rib space where the mud dried first (as a surrogate measure of the most metabolically active site). Modern imaging has since superseded this mud-guided approach; however the same challenges on drainage remain. Pleural infection is often characterized by extensive septations partitioning infected fluid into multiple locules that prohibit complete fluid evacuation.[10] Surgery is often required, either as first line treatment or when chest tube drainage failed, to facilitate breakdown of adhesions to empty pleural pus. However, surgery is expensive and has risks especially in this subgroup of patients who are often elderly with comorbidity.

Fibrinolytics (eg streptokinase, tPA and urokinase) activate plasminogen to generate plasmin that can lyse pleural adhesions.[11,12] Sherry and Tillett first pioneered the concept ~60 years ago that intrapleural fibrinolytic therapy (IPFT) could clear the transitional fibrin neomatrix that contributes to fibrinous pleural adhesions, and enhance drainage and improve outcomes of empyema.[13,14] IPFT was widely practiced for decades based on uncontrolled observational series. Clinicians' enthusiasm for intrapleural fibrinolytics in part stems from the 'reassurance' from the marked (up to 9-fold) increase in pleural fluid drainage following IPFT.[15] It only became apparent in recent years that fibrinolytics potently stimulates pleural fluid formation (discussed below) and can create a false impression of treatment success. The Multi-center Intrapleural Sepsis Trial (MIST)-1[6], the largest (n=454) randomized controlled trial (RCT) in pleural infection, as well as a single center RCT (n=54)[16], both found no benefit of intrapleural streptokinase (over placebo) in reducing surgical referral or death. This prompted a re-think of strategy.

Pus is thick because of liberated DNA from degranulated leukocytes. Simpson *et al* hypothesized that DNase can reduce pleural pus viscosity and improve drainage[9], similar to its role in thinning sputum in cystic fibrosis. He collected pus from empyema or abscesses in the community hospital

in Australia he was working in and built a simple device (Figure 1) to confirm that addition of DNase significantly improved pus flow.

The idea by Simpson *et al* was subsequently taken up by Light *et al* who applied the therapy in their validated rabbit empyema model.[17] The result from Light's work, also published in CHEST, showed that combination tPA/DNase therapy had synergistic effect in treating empyema. This finding was translated via the MIST-2 RCT (n=199) from UK centers which confirmed that combination tPA/DNase therapy significantly improved radiographic clearance, reduced need for surgery and shortened hospitalization when compared with each agent individually or with placebo (Fig 2).[7] Subsequent open-labelled series from Australasia, UK and USA all confirmed that intrapleural tPA/DNase therapy cures 90^{+}% of patients, making surgery now a rare event in the treatment algorithm (Fig 3).[18-20]

FUTURE DIRECTIONS

Optimization of Treatment and Extending Safety Profiles

Intrapleural tPA/DNase comes through an investigator-led pathway. This differs from most modern day therapies developed by pharmaceutical or device industries, in particular that there was no phase I study to establish the optimal dosing or long term pharmacovigilance follow-up.

The dosing regime used in MIST-2[7] was empirically chosen, and there are likely to be grounds for fine tuning. Various centers have already piloted simplifications of the treatment regime, including using a daily (instead of twice daily) dose[19], and administering the two drugs at the same time (rather than 45-60 min apart)[20]. Data from these longitudinal series also reinforced the high success rates of tPA/DNase and the short-term safety profile[18], despite the variations in regimes.

The mounting evidence of the efficacy of tPA/DNase therapy at its current regime[8], and the rapid uptake of its use worldwide, mean it is now difficult to justify performing a conventional phase I clinical trial using this approach. A pilot multi-centered dose de-escalation study is near completion; it assesses a pragmatic approach to begin therapy with a lower (5mg) tPA dose, and return to the conventional dose (10mg) if lack of clinical response is observed. If this study is successful, further de-escalation attempts may be warranted.

Both tPA and DNase have been used for decades and therefore toxicologic complications of the intrapleural combination of these agents are unlikely. However, the longer term effects of therapy, eg on lung functions, remain to be established.

In the era of personalized medicine, the question of individualizing the dose of IPFT to minimize bleeding risks and costs is attractive. Some centers based the dosing on the turbidity of the pleural fluid but such measure remains unproven. The "Fibrinolytic Potential" has been proposed by Komissarov and colleagues[21], in which the dose of fibrinolytics drugs is titrated to the plasminogen activator activity of freshly collected pleural fluids; the assumption being that patients with reduced plasminogen activator activity theoretically require higher dosing of fibrinolytics. The concept awaits clinical validation.

More Refined Fibrinolytic Agents

IPFT is subject to relatively rapid inactivation by inhibitors including antiplasmins and by plasminogen activator inhibitor-1 (PAI-1) that exist in pleural fluids.[11,12,22-26] Locally elevated PAI-1 occurs in loculations[22,25] and likely increases development of pleural septations. PAI-1 tends to rise with aging and haplotypes with increased PAI-1 expression are associated with higher susceptibility to community acquired pneumonia in elderly white patients[27]. Whether age-dependent changes in PAI-1 are associated with empyema is unclear.

However, PAI-1 can be targeted with mitigation of pleural injury in animals and adjunctive PAI-1-targeting monoclonal antibodies allow reduction in the dose of IPFT.[28] This strategy was designed to mitigate bleeding risk, and can be extended to treat other loculated (eg malignant) pleural effusions[29]. In a related vein, single chain urokinase (scuPA) has been manufactured through the NIH/NHLBI SMARTT program and is moving towards phase I dose escalation clinical trial testing in patients with loculated empyema. scuPA is a proenzyme fibrinolytic that is relatively slowly activated and inactivated by PAI-1 and generates durable, relatively low level intrapleural PAI-1-resistant PA activity[23]. Given intrapleurally, scuPA has been tested in rabbits with *S. pneumoniae* empyema and outcomes compared with tPA-treated controls. Using intrapleural doses that effectively clear adhesions in the tetracycline model of pleural injury in rabbits, both agents were well-tolerated but only scuPA was effective. These preliminary results suggest that the PAI-1

resistance and durability of intrapleural scuPA may be of advantage and raise the intriguing possibility that it could likewise prove even more effective when combined with DNase.

Manipulating the key molecules in the pleural fibrosis (eg transforming growth factor-beta)[30] has shown a glimpse of promise in providing alternative targets for adjunct therapies.

Interactions among tPA, DNase, bacteria and local inflammatory cells

It is postulated that tPA breaks adhesions while DNase reduces pus viscosity to enhance drainage. This is likely to be an over-simplistic view. The complex interactions of tPA and DNase with bacteria, antibiotics and cellular contents of the pleural cavity (i.e resident mesothelial cells and recruited inflammatory cells) are virtually unknown (Fig 4). Fibrinolytics, eg streptokinase, are not bacteriocidal[31] but urokinase may be able to reduce viscosity of pus[32]. Its activity may also be influenced by the quality and quantity of DNA in pleural fluids[33].

IMPLICATIONS BEYOND PLEURAL INFECTION

Medical regulation of pleural fluid formation

IPFT potently induces pleural fluid formation in significant quantities in human, rabbits and rodents, and in normal or diseased pleura, in a way not seen with other compounds[6,7,17,34,36]. Piccolo *et al* reported a median fluid output of 2.5 liters in 107 patients who received tPA/DNase; most of the fluid was likely to be induced by the therapy (Fig 5).[18] Understanding the mechanisms driving fibrinolytic-induced fluid synthesis may cast new knowledge on exudative fluid formation and novel therapy for exudative pleural fluid formation. It is possible that this observation may yet lead us to further breakthroughs beyond pleural infection.

Over 1500 people per million population develop an exudative pleural effusion a year; pleural infection and malignant pleural effusion (MPE) being the leading causes. Active plasma extravasation from juxtapleural hyperpermeable blood vessels is a cardinal mechanism of effusion development[29,37-43] and has been shown in animal models of various pleural effusions[44-50]. Moreover, inhibition of multiple tumor-to-host signalling events in mouse models of MPE resulted in dramatic reductions in pleural fluid accumulation without affecting tumor growth[51-55]. To date

control of recurrent symptomatic pleural effusion relies on pleurodesis and/or drainage devices (eg indwelling pleural catheters [IPC]), all of which have significant shortcomings. It has long been argued that targeting the mechanism of fluid to 'switch off the leaky faucet' is the ultimate goal for pleural research.[56]

Although the way by which bacteria and metastatic tumor cells trigger pleural fluid extravasation are most certainly distinct, it appears that both malignant and infectious pleural effusions share a significantly overlapping pleural inflammatory milieu, even displaying similar fluid kinetics[57,58]. This common inflammatory denominator, comprised by a multitude of cells and molecules, could be a marked target for the iatrogenic control of effusions (Fig 6).

The first common pathway to effusion development in infection and cancer appears to be the activation of proinflammatory transcriptional programs in mesothelial, immune, and cancer cells. Nuclear factor (NF)-κB is a master regulator of innate immune responses and is universally activated in mesothelial cells in all types of pleural effusion[59-65]. Activation of NF-κB in resident cells leads to enhanced transcription of hundreds of proinflammatory genes including CCL2, tumor necrosis factor (TNF), interleukins (IL), and osteopontin (OPN)[66,67]. Subsequently, these mediators activate the transcription factor in pleural resident or tumor cells in an autocrine or paracrine fashion (TNF, IL-1β, and others) or function to orchestrate myeloid and lymphoid cell recruitment to the pleural cavities (CCL2, OPN, and others)[51,68-70].

Inflammatory cells, once homed to the pleura, further enhance the activation of NF-κB, thus perpetuating inflammation[70,71]. Targeting NF-κB provides meaningful benefit to experimental mice by reducing their inflammatory effusions or MPEs. However, there are no safe and effective pharmacologic means to inhibit NF-κB[59-73]. Systemic NF-κB inhibition displays limited efficacy and, sometimes, severe toxicity[74,75]. This problem will likely be circumvented by targeting alternative pathways of NF-κB activations important in infection and cancer[76,77]. In addition to NF-κB, other transcription activation pathways are activated in pleural diseases, such as signal transducer and activator of transcription (Stat) 3, Notch, and phosphoinositide 3-kinase/protein kinase B, which may also present marked targets for therapy[59,60,63,70].

Activation of transcriptional programs in pleural resident cells during pleural infection and malignancy results in the local secretion of a cocktail of pro-inflammatory signalling molecules within the pleural confines. These mediators directly alter the function of endothelial cells that lie immediately under the mesothelial layers by inducing their proliferation and by loosening the

adhesion between adjacent endothelial cells, rendering the local vasculature leaky to plasma but also blood cells[38,43].

Monocyte Chemotactic Protein (MCP)-1, also known as CCL2, is such a mediator. CCL2 is a small cytokine that belongs to the CC chemokine family. CCL2 recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection. Recent researchers have shown that CCL2 is responsible for induction of vascular permeability in MPEs[54,69,78]. Recently Lansley *et al* found that tPA-induced pleural effusion is driven by MCP-1 and MCP-1 antagonists potently inhibits the large effusion formation following intrapleural fibrinolysis in mice[34]. CCL2 activity can be clinically modulated using monoclonal antibodies that have gained widespread use, but may cause systemic toxicities[78-80]. Intrapleural administration of such antibodies (eg via an IPC) would potentially circumvent systemic adverse events.

Another marked therapeutic target in this category of vasoactive mediators is osteopontin (OPN), which was recently shown to be a potent inducer of vascular leakage in malignant effusions, but is also highly expressed in infectious effusions[58,68]. Various methods to inhibit OPN signalling are in preclinical or clinical development, including antibodies, morpholino antisense oligonucleotides, and small molecule inhibitors[81-85], and will hopefully culminate in their clinical deployment against pleural effusions.

The above-discussed signalling molecules, as well as others reviewed elsewhere[50], may also enter the systemic circulation from the pleural effusion, with subsequent induction of a systemic inflammatory response characterized by increased circulating and pleural-homed myeloid and lymphoid cells[51,52,54,68-73,86-88]. These cells, found in most exudative effusion states including pleural infection and MPEs, sculpt the pleural inflammatory environment by either fuelling or switching off inflammation. The definition of their nature, phenotype, and exact role are of paramount importance, since their recruitment and activation can be modulated with emerging drugs. However, sparse cellular components of effusions may be keys to regulating pleural inflammation, such as was recently shown to be the case for mast cells.

Giannou *et al* recently discovered that, while only a few hundred mast cells reside in the normal pleura, tens of thousands are recruited locally during MPE development. Interestingly, tumor-elaborated CCL2 was the major mediator of the pleural homing of mast cells, and this phenomenon could be inhibited using anti-CCL2 antibodies, with marked beneficial effects in experimental models[70]. Tumor cells did not only chemoattract mast cells, but also triggered their degranulation

via OPN. Mast cell granules were found to be the major source of pleural fluid tryptase AB1, as well as IL-1β in this study, the former causing vascular leakage comparable to that induced by vascular endothelial growth factor (VEGF) and the latter activating NF-κB-based transcription in tumor cells[70]. This tumor-mast cell circuitry may exist in infectious and inflammatory effusions, and may be effectively targeted in humans in the future, as was the case in experimental animals enrolled in the above study that responded favorably to imatinib mesylate and CCL2 neutralization.

Conclusions

Sir William Osler once stated "empyema needs a surgeon and three inches of cold steel, instead of a fool of a physician"[89] and underwent rib resections for his own empyema. A century later, intrapleural tPA/DNase therapy has made surgery unnecessary in the majority of cases. The accelerated understanding of the fundamental mechanisms of effusion development and of fibrinolytic functions gained over the past decade combined with recent improvements in pleural procedures and techniques have begun to revolutionize clinical practice in pleural effusion care. Further translational research is needed for the development of novel etiologic therapies aimed at targeted interventions into effusion formation and resolution.

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Figure Legends

Figure 1. *Dr Simpson and his fellow built a simple home-made device with a syringe and needle nailed to a board to test the efficacy of DNase. The time for the pus to run from the top of the syringe to the end of the needle was recorded. Pus mixed with DNase +/- streptokinase ran significantly faster than controls.*[9]

Figure 2. *These two graphs showed the striking resemblance between the control groups (red) and tPA/DNase group (green box) in the rabbit experiments (LEFT) and MIST-2 RCT (RIGHT) where tPA/DNase resulted in a lower empyema score and more radiographic clearance respectively.*[7,17]

Figure 3. *This patient had a recent cholecystectomy for acute cholecystitis related to gall-stones. She had underlying liver cirrhosis, chronic myelomonocytic leukemia and mild pancytopenia. She re-presented after discharge with fever and a multi-loculated right-sided pleural infection which failed to improve with intravenous antibiotics, and was transferred to our unit for further management.*

(Left) A chest tube (18F) was inserted into the basal collection but failed to evacuate the fluid.

(Middle) Instillation of intrapleural tPA/DNase (three doses) via the chest tube provided rapid clearance of the basal collection. Her leucocytosis and high inflammatory markers improved but remained elevated. A second chest tube (12F) was inserted into the remaining posterior mid-zone collection. Again the fluid failed to be evacuated because of multiple loculations within the collection.

(Right) Instillation of tPA/DNase into the mid-zone collection completely cleared the residual collection and the patient made an excellent recovery.

Figure 4 A & B. The complex interactions of bacterial infection within the pleura with inflammatory responses and clinical interventions (antibiotics and tPA/DNase).

(A) *Pleural infection usually leads to effusion formation and loculations which are in part contributed by the initial responses of circulating inflammatory cells (1). Treatments to resolve the infection and increase ease of drainage include antibiotics (2) and tPA/DNase (3). Complex interactions exist between various elements above, as well as the bacteria involved. Our understanding of these interactions is extremely limited at present. Current studies now show that these treatments may have further implications in pleural effusion formation.*[34,35]

(B) *Complex interplay of the intrapleural fibrinolytic drugs and their potential effects on the pleural adhesions, as well as unresolved issues on their actions.*

Figure 5. *Intrapleural tPA therapy is typically followed by the drainage of a large volume of hemorrhagic pleural fluid.*

Figure 6. Schematic representation of pleural effusion development in pleural infection and cancer. *Resident (mesothelial cells and macrophages) and alien (tumor cells) cells in the pleural space activate transcription of critical factors such as nuclear factor (NF)-κB, signal transducer and activator of transcription (Stat) 3, and Notch upon bacterial and cancerous pleural invasion, resulting in enhanced secretion of cytokines and chemokines into the pleural cavity and, subsequently, into the bloodstream. Increased pleural and systemic mediator levels result in enhanced vascular permeability, as well as in the recruitment of myeloid and lymphoid cells to the pleural space, with the latter contributing secondarily to the inflammatory and vasoactive phenomena that ultimately lead to effusion development.*