Bleomycin revisited: towards a more representative model of IPF?

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TRANSLATING BASIC SCIENTIFIC discoveries to the clinic remains one of the most challenging problems in biomedicine. A prerequisite for translational research is the availability of an animal model that sufficiently recapitulates the hallmark characteristics of the disease and in which interventional studies are predictive of success in the clinic. Animal models are also commonly used for improving our understanding of the pathomechanisms of disease and for practical assessment of the pharmacodynamic and pharmacokinetic properties of potential novel pharmacological interventions. Idiopathic pulmonary fibrosis (IPF) is a progressive fibrolproliferative disorder refractory to current pharmacological therapies with a median survival of only 3–5 years following diagnosis (6). Yet it is widely accepted that the most commonly used experimental model of IPF, based on the instillation of bleomycin, fails to reflect many of the histological and pathological features typical of the human disease.

The earliest studies investigating bleomycin-induced pulmonary fibrosis in experimental systems date back to the early 1970s, using guinea pigs and dogs (7, 17), with the first studies in mice published in 1974 (1). Today, a literature search for “bleomycin+fibrosis+mouse” in PubMed would yield upwards of 700 papers since 1974. Recently, Degryse et al. (5) described a refinement to the bleomycin model, involving an 8 biweekly dosing regimen with mice killed 2 wk following the last dose, instead of the commonly used single challenge model. Although mortality is relatively high (33%) and an experiment will require a time commitment of more than 4 mo, this regimen results in a persistent fibrosis (at least out to 70 days after the last administration of bleomycin). Importantly, these authors report that this schedule leads to prominent type II alveolar epithelial cell (AECII) hyperplasia as well as the emergence of (rare) fibroblastic foci, a major historical feature of usual interstitial pneumonia and commonly believed to represent the highly dysregulated epithelial-mesenchymal cross talk in this condition.

Under normal circumstances, epithelial-mesenchymal cross talk forms the basis for an appropriate tissue response to injury, following which wound healing can progress normally to restore tissue architecture with limited loss of function. The pathogenetic mechanisms of IPF remain incompletely understood and highly debated within the IPF research community. However, there is now increasing evidence that recurrent damage to the alveolar epithelium, and the ensuing dysregulated epithelial-mesenchymal cross talk, leads to an inappropriate expansion and activation of the mesenchyme. This aberrant wound healing response leads to the characteristic excessive deposition of extracellular matrix, epithelial hyperplasia, and the subsequent impairment of normal respiratory function (9). A vast number of studies have investigated the underlying mechanisms for this dysregulated wound response in IPF, highlighting that an imbalance in profibrotic mediators (such as TGF-β, IL-13, coagulation factors, endothelin, etc.) vs. anti-fibrotic mediators (such as PGE2 and IFNy) might be at the core of the problem (14). More recent studies have highlighted the potential role of epithelial-mesenchymal transition (EMT), recruitment of circulating mesenchymal progenitor cells (fibrocytes), and the involvement of resident lung progenitor cells (20). The role of inflammation in IPF remains a highly debated issue and is perceived to be a major weakness of the bleomycin model, since the ensuing fibrotic response following bleomycin instillation is highly dependent on the early inflammatory response in this model (3). Overcoming this limitation of the bleomycin model was not the initial focus of the current study by Degryse et al. (5), but what their refinement might offer over the standard single-dose version is a much improved opportunity to study epithelial-mesenchymal interactions, EMT, and the genesis of AECII hyperplasia.

It is worth mentioning that while bleomycin may be the most commonly used model of experimental lung fibrosis, we would argue that that there is anything but a “common” form of the model, and that despite its widespread use there remains a significant number of misconceptions and discrepancies regarding the natural history of the mouse lung response to bleomycin. Historically, bleomycin has been administered to experimental animals (normally juveniles) intravenously, intraperitoneally, subcutaneously (including sustained delivery via osmotic minipumps), and by direct instillation into the respiratory tract, with the latter route being the most widespread. Doses vary considerably in both quantity and units (U/mouse, U/kg, or mg/kg), although values around 1–2 mg/kg (roughly equivalent to 0.025–0.05 U/mouse) are frequently used. Moreover, there are considerable strain-dependent differences in the fibrotic response, with C57Bl/6 mice being more susceptible than Balb/c, for example (19). Even with direct instillation into the lungs, there are hugely variable responses that may be generated depending on the technique used (surgical tracheostomy, oropharyngeal instillation, intubation, or microspray). Clearly, therefore, a bewildering number of permutations and combinations exist for the bleomycin model. In our laboratory, we classically used surgical tracheostomy followed by injection of bleomycin (0.025 U/mouse) directly into the trachea, which resulted in a robust fibrotic response at 2 wk postbleomycin, with a bias towards a bronchio-centric distribution (8, 10). More recently, we (21) have employed the method of oropharyngeal instillation proposed by Lakatos et al. (12). The latter approach has improved the homogeneity of our fibrotic responses, including a more peripheral, subpleural fibrosis, which extends to the base of the lungs. Therefore, even within a single laboratory, we have two markedly different versions of the murine “bleomycin model.”

In terms of disease progression, intratracheal instillation generally results in an early inflammatory response (due to widespread damage to the epithelium, combined with vascular...

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leak, upregulation of proinflammatory cytokines and chemokines, and recruitment of inflammatory cells), which is more akin to acute lung injury/ARDS rather than IPF. During the second week postbleomycin, there is a transition to the fibrotic phase which peaks at around 3–4 wk. This phase is characterized by expansion of the myofibroblast population, increased deposition of extracellular matrix, and an overabundance of profibrotic cytokines such as TGF-β (3). The time to onset of murine fibrosis itself advises caution, given that the human disease likely develops over a timespan of decades. As mentioned above, certain histological features that are characteristic of IPF, such as fibroblastic foci and hyperplastic AECII, are widely assumed to be absent in the single-dose model.

After this, the reported lung responses are a little more discrepant, leading to perhaps one of the most crucial limitations of the model, which sets it apart from being truly representative of IPF: the issue of resolution or lack of progression of fibrosis. Numerous publications state that the fibrosis resolves, and there is evidence to support this; Chung et al. (4) and Degryse et al. (5) show almost complete resolution of fibrosis 6 wk following single-dose bleomycin injury. In contrast, work from our laboratory has demonstrated that the increased deposition of lung collagen peaks at 28 days post-bleomycin and is maintained out to 3 mo (8). Therefore, depending on the particular permutation of the model, the fibrosis may be resolved or maintained. There is little disagreement, however, that the fibrosis does not ordinarily progress, and this may be a sufficient stumbling block that prevents the bleomycin model (whichever version it may be) from being a robust indicator of future clinical outcomes. The recommendations from the recent excellent systematic review of drug efficacy studies in the single bleomycin dose model by Moeller et al. (15) also deserve further mention here. It is critical to distinguish between drugs interfering with the inflammatory and early fibrogenic response from those preventing progression of fibrosis, the latter likely much more meaningful for clinical application. Potential antibifromic compounds should therefore be evaluated in the phase of established fibrosis rather than in the early period of bleomycin-induced inflammation for assessment of their antibifromic properties.

If not bleomycin, what are the alternative models that might be worth considering? A plethora of fibrosis models exist: FITC, radiation, amiodarone, silica, direct AECII injury (22), and TGF-β overexpression, to name a few (reviewed in Ref. 16), but they all have specific advantages and disadvantages. Given that IPF is thought to arise due to repetitive epithelial injury, the regimen adopted by Degryse et al. (5) is logical. A similar approach has been tried before by various routes (intravenous, subcutaneous, and intratracheal). One of the earliest studies dates back to 1988, when Brown et al. (2) gave repeated doses of intratracheal bleomycin to rats. In that study, three doses of bleomycin given 1 wk apart were sufficient to cause a progressive increase in extent and severity of fibrosis (at least out to 90 days after the final dose of bleomycin), rather than the gradual regression seen after a single dose. Similar findings were reported by Pinart et al. (18) in terms of biomechanical changes to the rat lung in a comparable repetitive dosing model. Needless to say, the story in mice is less consistent: in the study by Chung et al. (4), the fibrotic change following repetitive dosing (3 doses, one week apart) was fundamentally resolved 6 wk following the final dose. However, in the current refinement of the repetitive model, Degryse et al. (5) report persistent fibrosis that had not regressed by 10 wk following the final dose.

The presence of extensive AECII hyperplasia has allowed Degryse et al. (5) to further investigate the role of bronchoalveolar stem cells in lung repair, as well as the notion of epithelial cell plasticity and EMT that may underlie the development of fibrosis. The degree to which EMT contributes to the fibroblast pool may be as high as 50% in this repetitive dosing model, which may in itself represent an ideal opportunity to investigate and indeed interfere with a process that is theorized to be relevant in the human disease. Ongoing studies will undoubtedly aim to further characterize the fibrotic changes in this repetitive model, and particularly the contribution of AECII hyperplasia to fibrotic progression.

Which brings us to the question: Where next for the bleomycin model(s)? We believe it is generally agreed that we understand the pathogenesis of experimentally induced pulmonary fibrosis better than we do the human disease. It could be argued that we still have a lot to learn about the current gold standard model. The various permutations of strains, dosing regimens, routes of administration, times of death, measures of fibrotic outcome, and scheduling of therapeutic interventions mean that a standard bleomycin model does not exist. Data comparisons between studies can therefore prove problematic. One laboratory may use a model that resolves after 6 wk, another where fibrosis is maintained for 3 mo. However, this recently much maligned model still has a lot to offer, particularly with refinements such as presented by Degryse et al. (5). The study of both the genesis and the contribution of AECII hyperplasia during the fibrotic response will now be eminently enabled in this model. The next challenge will be to establish whether further refinements can generate the robust and frequent occurrence of fibroblastic foci, currently held to represent the leading edge of fibrogenesis during the development of IPF (11). It is possible that this histological feature may depend on the slow development of the disease over many decades coupled with a uniquely altered fibroblast phenotype (13), circumstances that may not be easily achievable within the context of the bleomycin model. Nevertheless, with careful study design, we believe that the bleomycin model still has much to offer both in terms of informing on the cell and molecular mechanisms involved in driving fibrosis and in terms of evaluating novel targets for therapeutic intervention.

DISCLOSURES

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