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Challenges for inhaled drug discovery and development: Induced alveolar macrophage responses ☆

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ABSTRACT

Alveolar macrophage (AM) responses are commonly induced in inhalation toxicology studies, typically being observed as an increase in number or a vacuolated 'foamy' morphology. Discriminating between adaptive AM responses and adverse events during nonclinical and clinical development is a major scientific challenge. When measuring and interpreting induced AM responses, an understanding of macrophage biology is essential; this includes 'sub-types' of AMs with different roles in health and disease and mechanisms of induction/resolution of AM responses to inhalation of pharmaceutical aerosols. In this context, emerging assay techniques, the utility of toxicokinetics and the requirement for new biomarkers are considered. Risk assessment for nonclinical toxicology findings and their translation to effects in humans is discussed from a scientific and regulatory perspective. At present, when apparently adaptive macrophage-only responses to inhaled investigational products are observed in nonclinical studies, this poses a challenge for risk assessment and an improved understanding of induced AM responses to inhaled pharmaceuticals is required.

Abbreviations: 3D, Three dimensional; AM, Alveolar macrophage; APSGB, Academy of Pharmaceutical Science of Great Britain; BAL, Bronchoalveolar lavage; CAD, Cationic amphiphillic drugs; COPD, Chronic obstructive pulmonary disease; DLCO, Diffusing capacity of the lung for carbon monoxide; ERK, Extracellular signal-related kinase; FDA, Food and Drug Administration; FFPE, Formalin-fixed paraffin-embedded; H&E, Hematoxylin and eosin; HESI, Health and Environmental Sciences Institute; HIV, Human immunodeficiency virus; IL, Interleukin; INF, Interferon; iNOS, Inducible NO synthase; IM, Interstitial macrophages; LPS, lipopolysaccharide; MAP, p38 mitogen-activated protein; MDM, Monocyte derived macrophage; MMP, matrix metalloprotease; NHP, Non human primate; NOAEL, No observed adverse effect level; OSWG, Oligonucleotide Safety Working Group; PAP, Pulmonary alveolar proteinosis; PCR, Polymerase chain reaction; PFT, Pulmonary function test; PK/PD, Pharmacokinetics/pharmacodynamics; PM, Particulate matter; STP, Society of Toxicologic Pathologists; TGF, Transforming Growth Factor; TNF, Tumor necrosis factor.

☆ This article is based upon an international workshop held by the Academy of Pharmaceutical Sciences Great Britain and Health and Environmental Sciences Institute on 30–31 October 2012. The meeting addressed the challenge of induced alveolar macrophage responses facing those undertaking inhaled product development. Details of the workshop program, participants, presentations, discussions and the consensus achieved are freely available on the APSGB website http://www.apsgb.co.uk/FocusGroups/DrugsInTheLungs/. This article by the meeting organizers and expert speakers aims to deliver a more detailed perspective on the topics discussed and conclusions reached at the meeting.

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1. Introduction

Delivery of drugs by inhalation has a proven track record for safe and effective treatment of human respiratory diseases, principally asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis and infection [1,2]. The development of new and improved inhaled medicines, however, presents a number of challenges that have been reviewed previously [3]. This article considers induced alveolar macrophage (AM) responses, the interpretation of which is a significant challenge for safety assessment in inhaled product development. The commentary is based on a workshop held in October 2012, organized by the Academy of Pharmaceutical Science of Great Britain (APSGB) 'Drugs in the Lungs Network' in collaboration with the Health and Environmental Sciences Institute (HESI). This meeting comprised a series of structured debates which were led by the authors and informed by workshop participants [4]. In accordance with the principles of the APSGB and HESI organizations, the report reflects multisector perspectives and emphasis is given to the scientific developments and collaborative approaches required for a more efficient paradigm for developing inhaled medicines.

1.1. Safety challenges in developing inhaled medicines

Compound failures during development are costly and contribute to the industry-wide high rate of attrition during drug development [5]. Safety is an important cause of attrition during the development of inhaled medicines. For example, AstraZeneca reported at the workshop that over the last seven years safety was the second-most common reason (30% of 33 cases) for halting further development of inhaled compounds (small molecules targeted at local activity in the lung) which had reached the stage of repeat dosing with a range of doses in one or more species in nonclinical studies. Others have suggested that safety failures may be, in part, because the design considerations for improved lung-targeted medicines (*i.e.*, high molecular weight, lipophilic compounds) have resulted in poorly soluble compounds which generate lung pathology findings related to an excess of undissolved drug [6]. As new classes of molecules are developed as inhaled medicines, including biopharmaceuticals, compounds for new targets in the lung or for systemic delivery *via* inhalation, and compounds requiring novel advanced delivery systems such as nanoparticle or liposomal systems, safety assessment may provide greater challenges [7].

Regulatory guidelines dictate well-defined nonclinical (formerly referred to as preclinical) and clinical phases of inhaled medicine development [8]. At present, Good Laboratory Practice inhalation toxicology studies supporting clinical trials utilize histopathological examination of hematoxylin and eosin (H&E) stained tissue sections as the primary endpoint [9,10]. The most common responses to aerosol administration in nonclinical studies are nasal and laryngeal irritation in rats, which are generally accepted to have little relevance for human orally inhaled drug products as they result from obligate nasal breathing and species-specific airway geometry, respectively [11]. Lung irritation, observed in acute studies as changes to the epithelium at the bronchial or alveolar level (*i.e.* epithelial degeneration, ulceration, necrosis) may be seen as a high-dose effect in short-term studies. However, these effects are rarely seen with chronic dosing as doses are likely to be lower or the drug will already have been discontinued without progressing to long term toxicology studies if this occurs at lower doses.

Lung histology typical of that observed in nonclinical studies is illustrated in Fig. 1. The significance of the common histology finding of an increase in macrophage numbers in the lung and/or alterations in macrophage morphology is not clear. The challenge to toxicologists, pathologists, clinicians and regulatory scientists is to determine at what point a normal adaptive response to foreign inhaled materials becomes a pathological process in animals and at what point the response is predictive of a potentially adverse consequences for treated patients. One complication is that AM responses are often seen in control groups. For example, analysis of control animals in nonclinical studies revealed macrophage accumulations as spontaneous findings in air-only control cynomolgus monkeys in 32 cases, 5.6% of animals; range of 0–40%, in 55 studies [12]. Another concern is that the inhaled medicine is most often for the treatment of respiratory disease, *i.e.* patients who *de facto* have underlying lung pathologies and may be more sensitive than healthy animals or human volunteers to inhaled particles.

A continuum of responses involving AMs can be recognized in association with the nature, degree and duration of inhaled stimuli. This spectrum of histological findings extends from minimal increases in AMs disseminated within the pulmonary parenchyma, through gradually escalating numbers and densities of AMs, sometimes associated with hypertrophy. Such changes are graded by pathologists (*e.g.* minimal, mild, moderate, etc.) in order to facilitate comparison between treated and control groups. It has been proposed that simple increases in qualitatively similar AMs typically constitute adaptive, physiological responses that are not adverse [6]. In contrast, some stimuli, such as drug accumulation above a certain level, may drive pathologic, adverse responses involving AMs in association with combinations of other changes including infiltrations of inflammatory cells (*e.g.* neutrophils, lymphocytes), epithelial and interstitial changes, and fibrosis [11,13].

While it is generally assumed that certain responses to inhaled particles constitute a normal physiological response that is reversible and distinct from a pathologic response, at present there is no clear agreement for determining where this threshold occurs and how it can be defined objectively using available methodologies. This uncertainty can lead to delays or non-approval for a drug to enter clinical studies and can place a limit on the doses that can be evaluated clinically. A question raised previously [3] is whether toxicological data are obtained and reported similarly between companies or is inconsistent reporting of histopathology findings creating a more complex picture than necessary? The diagnostic criteria published by the INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) initiative of the North American, European and Japanese toxicological pathology societies should assist in partially alleviating this concern [14]. Proposed refinement of the INHAND terminology specifically to address increases in alveolar macrophages when they are observed in nonclinical studies of inhaled pharmaceutical compounds [15]may further promote consistency in reporting results.

1.2. Regulatory considerations

Nonclinical toxicology studies required to support the development of inhaled drugs are generally the same as for other routes of administration [11]. Development plans usually follow the recommendations outlined in the relevant International Conference on Harmonization guidelines [8]. Where possible, repeat dose toxicology studies should employ the inhaled route of administration to mimic the intended clinical route of administration and to ascertain any potential for adversity.

Due to the high frequency of induced AM responses in nonclinical studies, any developer of inhaled drugs is likely to have observed test article-related increases in macrophage numbers, considered the impact of this for their clinical program and engaged in dialogue with the US FDA or other regulatory agency. Regulatory guidance specific to interpretation of alveolar macrophage responses is not currently available. Interpretation of an inhaled drug-induced macrophage response is an important consideration with regard to authorizing progression of products from nonclinical to clinical phases, especially in terms of trial dose and duration, and for clinical indications involving lung disease. The nonclinical/clinical interface is where the interpretation of adversity is critical. If the principle that any increase in macrophage numbers should be considered a potential early indication of inflammation is applied, due to the lack of a monitoring tool in clinical studies, this impacts on the determination of the 'no observed adverse effect level' (NOAEL). A lower NOAEL value, in turn, affects safety margin calculations required for transfer to the clinic, thus limiting both the starting and maximal allowable dose in human trials, potentially

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