BACK PAGE

The Association of Inhalation Toxicologists Workshop at DDL2023: Preclinical Medicines Development—Back to Basics

Ben Forbes[a], Jo Kilgour[b] and Simon Moore[c]
On behalf of the Association of Inhalation Toxicologists
[a]King's College London, Institute of Pharmaceutical Science, London, UK
[b]Regulatory Science Associates, Mereside Toxicology Consulting and Apconix, UK
[c]Labcorp Early Development Laboratories Limited, Huntingdon, Cambridgeshire, UK

The Association of Inhalation Toxicologists (AIT) delivered a workshop at the Drug Delivery to the Lungs Conference (DDL2023) in Edinburgh on December 7, 2023. The aim was to educate academic and industry inhalation scientists involved in developing new inhaled drug molecules and formulations about inhalation toxicology. Regulatory safety requirements provide a common challenge for all inhaled medicine developers and the meeting brought together 140 members of the scientific community to learn about the methods used for *in vitro* toxicity screening, *in vivo* study designs, toxicological endpoints and regulatory toxicology. The workshop also covered technical aspects of the generation, characterization and dosimetry of aerosols in toxicology studies.

The AIT 2024 Annual Conference, "Inhalation Toxicology Rocks," will take place during the week of September 16 in Cleveland, Ohio, US, home of the Rock and Roll Hall of Fame.

Inhaled medicines development

Carsten Ehrhardt (Trinity College Dublin) reminded the audience that inhalation has been used as a means of drug administration for centuries. There are many advantages of drug delivery to the lungs including bypassing first pass metabolism, rapid therapeutic effects, local targeting for respiratory disease indications and, of course, reduction of systemic side effects. However, inhaled medicines development is not without challenges: it is technically difficult, can be more expensive than other routes of administration, requires complex device and formulation choices, is associated with poor patient compliance and has a high bar for commercialization-reimbursement. Despite these challenges, there has been a recent upsurge of interest in inhaled drug development for traditional respiratory disease targets and newer ones, such as interstitial lung disease and cancer (this includes the local delivery of inhaled biologics and vaccines), as well as a growing pipeline of inhaled drug candidates for systemic indications.

In vitro inhalation toxicology

Victoria Hutter (ImmuOne and the University of Hertfordshire) provided an overview of *in vitro* toxicology that covered the cell models available. These range from models based on single-cell types (including primary cell cultures and cell lines) to complex organotypic models that more faithfully represent the complexity of the respiratory tract. These models can be cultured under liquid submerged or air interface conditions. The importance of choosing the right model, selecting appropriate endpoints and designing experi-

ments meticulously to investigate the study question was emphasized. Multiple endpoints can often be used that combine signs of epithelial health (e.g., histology, cell/barrier integrity, ciliary beat frequency or individual cell characteristics), indicators of stress (e.g., inflammatory cytokines) and mechanisms of toxicity (e.g. signaling pathways).

Exposure mode and "dose" are key considerations for *in vitro* studies; for example, the amount and concentration of drug or formulation (whether administered in solution or by aerosol) and how drug levels relate to exposure scenarios *in vivo*. Establishing *in vitro/in vivo* correlation is important to support translation from cell and tissue experiments into animal testing, and the use of a well-characterized test article is an essential factor through all development phases to ensure that preclinical outcomes translate into the clinic.

Delivery of aerosols for inhalation toxicology

An(Tony) Grasiewicz (LabCorp) addressed the delivery of test materials in different respirable forms, focusing on liquid droplet and powder aerosols that are relevant to inhaled medicines development. The principle is to generate an atmosphere that is respirable for the species in the study and allow the animals to inhale it. While it is sometimes possible to use clinical devices, they are designed for humans and it is more common not to utilize them. Liquid formulations delivered by nebulization are commonly used in early development. For pressurized metered dose inhaler (pMDI) formulations, atmospheres can be produced by actuating multiple canisters simultaneously and repeatedly. For powder formulations, aerosols can be generated using capsule-based or bulk powder systems. Characterization of the atmosphere in terms of both aerosol concentration (mass per volume) and particle size distribution is key for calculating dose and determining the exposure achieved.

There are a variety of ways to expose the species commonly used in inhalation safety studies to aerosol atmospheres. For drug development studies, administration is typically by nose-only cones or masks for rodents, dogs, minipigs and non-human primates. Training and habituation of the animals to the experimental set-up is pivotal from both ethical and scientific perspectives.

Inhalation safety study designs and preclinical disease models

Paul Smith (Charles River) explained the regulatory toxicology requirements that apply to inhaled medicines. Toxicology safety study designs are largely driven by ICH M3 (R2) [1] or S6 (R1) [2]. Non-clinical studies for pharmaceuticals are designed such that the duration varies according to the planned duration of studies in the clinical development phases. In addition to the usual inhalation toxicology outcomes (e.g., clinical signs, body weight, food consumption, toxicokinetics, lung weight and histology), additional endpoints may be required for inhaled biologics. It is also useful to incorporate safety pharmacology endpoints into inhalation toxicology studies, if possible, as this provides an opportunity to evaluate effects after repeated dosing using a clinically relevant route of exposure.

Inhaled toxicology studies are usually more expensive compared to other routes of exposure and require more active pharmaceutical ingredient (API), due to inevitable losses during aerosol generation and delivery. Regulatory toxicology generally requires studies using a rodent and non-rodent species. Lung dose is calculated based on detailed characterization of tightly-controlled delivered test atmospheres (concentration and particle size) using the "Alexander equation" [3]. Target doses can be achieved by adjusting the aerosol concentration and duration of administration but there are limits for each based on practical and ethical considerations. For lung delivery, a 10-fold or 4-6-fold safety margin (above the clinical dose) is required for rodent and non-rodent species, respectively, and a 1-2-fold margin is required for achieved systemic exposure. There is inconsistency in approach between regulatory agencies for safety margin calculations for locally targeted exposure, with the US Food and Drug Administration (FDA) being the most cautious. In terms of translation to the clinic, the dose which causes no adverse effects (i.e., the no observable adverse effect level or NOAEL) in Good Laboratory Practice toxicology studies, together with application of the safety margin, are what determines the Maximum Recommended Starting Dose for First-In-Human trials.

Unlike safety studies that use healthy animals, efficacy studies often require a disease model in which to demonstrate therapeutic effects. Well-established respiratory disease models include:

- acute pneumonia (infection model),
- neutrophilia (lipopolysaccharide model for adult respiratory distress syndrome, cystic fibrosis and chronic obstructive pulmonary disease),
- fibrosis (bleomycin model for idiopathic pulmonary fibrosis),
- asthma (ovalbumin model using the Norway brown rat).

Two examples were discussed in more detail:

- Pneumonia—*Pseudomonas aeruginosa* is a major cause of ventilator-acquired pneumonia and a risk factor for adult respiratory distress syndrome. Rats were administered a clinical strain of *Pseudomonas aeruginosa* by oropharyngeal aspira-

tion followed by an aerosolized antibiotic (amikacin or Fosfomycin) 1-hour post-infection with efficacy demonstrated by change in temperature or reduction in bacterial load as colony forming units (CFU),

- Neutrophilia—a lipopolysaccharide-induced model of acute pulmonary neutrophilia in rats was described, in which dexamethasone (an experimental positive control) reduced both lung neutrophil count and the elevated cytokine levels in bronchoalveolar lavage fluid.

Novel excipients for inhalation and nasal toxicology

In response to pre-workshop feedback, Jo Kilgour (Regulatory Science Associates, Mereside Toxicology Consulting and Apconix) addressed two additional topics: novel excipients for inhalation and preclinical nasal toxicology studies.

Regulatory guidelines for qualifying an excipient are available. If a new excipient (or variation of type/concentration of an existing excipient) is for long term use, a 6-month rodent toxicity study is generally required; in addition, carcinogenicity studies (at least one of which should be by the relevant clinical route) will be conducted with the formulation for Marketing Authorization). In such studies, having an air control is paramount and multiple novel excipients can be tested in a "to-be-marketed" formulation. The advice given was to keep formulations simple and seek guidance on safety data requirements from the appropriate regulatory authority.

Nasally administered products have different safety considerations compared to orally inhaled products, both in terms of dose and safety margin. Practicalities dictate that dose is limited by nasal cavity size and the maximum volume and frequency of delivery allowable for each species, while the administration method (pipette, syringe, micro-sprayer) will be influenced by the nature of the test material.

Panel discussion

The workshop chairs, Ben Forbes (King's College London), and Viktoria McDonald (AlbaTox Consulting Ltd), facilitated a panel debate to complete the workshop. Delegates were curious about the following aspects of the topics discussed in the presentations: 1) whether the aerosol concentration and particle size need to be tailored to the test species or match the clinical aerosol characteristics, 2) what quality of test material is acceptable to support GLP toxicology studies, 3) how and when aerosol characterization, including API stability, should be performed over the course of a study, and 4) how long it will be before *in vivo* toxicology can be replaced by *in vitro* methods for drug development and accepted by regulators, particularly considering the FDA modernization act and approaches developed for the chemical/agricultural industry.

The content of this workshop is being supplemented by reference to the literature and will be published in 2024 as a review article titled, "Non-clinical toxicology for inhaled and nasally administered excipients and drug products: Regulatory requirements, current practice, emergent methodology and clinical translation." It will appear in *Expert Opinion on Drug Metabolism and Toxicology.*

The AIT techno-regulatory workstreams

Jo Kilgour, AIT President since 2011, also provided a brief history of the Association of Inhalation Toxicologists. The Society was established and held its first meeting in May 1981 and has held an annual meeting every year since, cycling between the UK, continental Europe and US. The Society has a number of workstreams and has been responsible for a variety of impactful publications [3-6]. Current article topics under active consideration include:

1) regulatory harmonization,
2) new approach methodologies (NAMs) for inhalation,
3) dosing approaches: time vs. concentration,
4) aerosol particle size distribution considerations and
5) AIT statement group on cannabinoids.

2024 Annual Meeting: Inhalation Toxicology Rocks

The next AIT annual meeting will take place in Cleveland, Ohio, US during the week of September 16, 2024. Topics will include:

- NAMs/*in vitro* toxicology (co-sponsored by The American Society for Cellular and Computational Toxicology; EU 2020 Horizon project HARMLESS),
- inhaled biologics/cell and gene therapy,
- aerosol generation *in vivo/ in vitro,*
- computational disease models,
- intranasal studies,
- wildfires and smoke inhalation toxicology.

For more information and to register: https://www.aitoxicology.org/conference-announcement.

References

1. ICH Harmonised Tripartite Guideline M3 (R2). Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals. https://www.ema.europa.eu/en/ich-m3-r2-non-clinical-safety-studies-conduct-human-clinical-trials-pharmaceuticals-scientific-guideline.

2. ICH Harmonised Tripartite Guideline S6 (R1). Preclinical Safety Evaluation of Biotechnology Derived Pharmaceuticals. https://www.ema.europa.eu/en/ich-s6-r1-preclinical-safety-evaluation-biotechnology-

derived-pharmaceuticals-scientific-guideline.

3. Alexander DJ, Collins CJ, Coombs DW, et al. (2008) Association of Inhalation Toxicologists (AIT) Working Party for Standard Delivered Dose Calculation and Expression in Non-Clinical Aerosol Inhalation Toxicology Studies with Pharmaceuticals. Inhalation Toxicology, 20:1179-1189.

4. Jones, DR, Baldrick P. (2013) Association of Inhalation Toxicologists (AIT) Review of Regulatory Aspects for Inhalation Toxicology Studies. Inhalation Toxicology, 25:84-90.

5. Forbes B, O'Lone R, Allen PP, et al. (2014) Challenges for Inhaled Drug Discovery and Development: Induced Alveolar Macrophage Responses. Advanced Drug Delivery Reviews, 71:15-33.

6. Wolfreys A, Kilgour D, Moore S, et al. (2021) Review of the Technical, Toxicological, and PKPD Considerations for Conducting Inhalation Toxicity Studies on Biologic Pharmaceuticals—The Outcome of a Cross-Industry Working Group Survey. Toxicologic Pathology, February;49(2):261-285.

For more information, please contact the corresponding authors: Ben Forbes, ben.forbes@kcl.ac.uk, Jo Kilgour, joanne.kilgour@regulatoryscience.com and Simon Moore, simon.moore@labcorp.com.